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Reviews in cardiovascular pharmacology 2023

Edited by
Yusof Kamisah, Mas Rizky A. A. Syamsunarno and Ismail Laher

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Reviews in cardiovascular pharmacology: 2023

Topic editors

Yusof Kamisah — Faculty of Medicine Universiti Kebangaan Malaysia, Malaysia
Mas Rizky A. A. Syamsunarno — Padjadjaran University, Indonesia
Ismail Laher — University of British Columbia, Canada

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Eliot Ohlstein,
Drexel University School of Medicine,
United States

*CORRESPONDENCE
Yusof Kamisah,
kamisah_y@hctm.ukm.edu.my

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Editorial: Reviews in cardiovascular pharmacology: 2023

Yusof Kamisah[1,2]*, Ismail Laher[3] and Mas Rizky A. A. Syamsunarno[4]

[1]Department of Pharmacology, Faculty of Medicine Universiti Kebangaan Malaysia, Kuala Lumpur, Malaysia, [2]Cardiovascular and Pulmonary University Research Group, Universiti Kebangaan Malaysia, Bangi, Selangor, Malaysia, [3]Department of Anesthesiology, Pharmacology and Therapeutics, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada, [4]Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

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Editorial on the Research Topic
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1 Introduction

Cardiovascular disease remains a leading cause of death globally, accounting for approximately 17.9 million fatalities in 2019 (World Health Organization, 2021). Cardiovascular diseases include hypertension, atherosclerosis, ischemic heart disease, stroke and heart failure, and represent a significant socioeconomic burden. The Research Topic *"Reviews in Cardiovascular Pharmacology: 2023"*provides an overview of both the pathogenesis and pharmacological advances in cardiovascular diseases. This Research Topic features 12 articles, each offering in-depth discussions of recent findings on the mechanisms underlying cardiovascular diseases, along with the development and application of novel cardiovascular therapies. The articles cover a wide range of topics that include recent advancements in clinical trials, emerging concepts in drug mechanisms, therapeutic strategies, and challenges related to pharmacokinetics.

Nitric oxide (NO) is a highly reactive gaseous molecule released by endothelial cells in blood vessels that plays a crucial role in mediating protective cardiovascular effects, for instance vasodilation (Siti et al., 2019). Impaired NO function often occurs before the clinical onset of cardiovascular (Abd-Elmoniem et al., 2024) disease. This endogenous vasodilator binds to soluble guanylate cyclase (sGC), stimulating the synthesis of cyclic guanosine monophosphate (cGMP). Elevated cGMP activates protein kinase G (PKG), which lowers intracellular calcium levels in vascular smooth muscle cells and induces vasodilation (Mishra et al., 2025). Yin et al. reviewed the progress of guanylate cyclase activators to stimulate the NO-sGC-cGMP signaling pathway in patients with cardiovascular disease. These include riociguat, vericiguat, praliciguat, olinciguat, cinaciguat, ataciguat, runcaciguat, mosliciguat, and BI 685509, the latter still undergoing clinical trials. Therapies such as riociguat and vericiguat show benefits such as improved quality of life in patients with heart failure and pulmonary hypertension, although definitive

conclusions for other agents are not yet available. Additionally, Yin et al. discussed the pharmacology of guanylate cyclase-C (GC-C) agonists (linaclotide and plecanatide) for the treatment of digestive conditions. These agents improve symptoms of irritable bowel syndrome with constipation (IBS-C) by activating the GC-C/cGMP pathway, reducing submucosal afferent neuron excitation and reducing abdominal pain.

While guanylate cyclase mediates its effects primarily through cGMP to cause vasodilation and smooth muscle relaxation (Siti et al., 2015), adenylyl cyclase produces cAMP to regulate diverse functions, such as metabolism, heart rate, and neurotransmitter release (Marsden and Dessauer 2019). Adenylyl cyclase isoforms 5 (AC5) and 6 (AC6) are highly expressed in cardiac tissues, where they are important in translating signals from β-adrenergic receptors into intracellular cAMP production (Marsden and Dessauer 2019). Maghsoudi et al. highlighted the distinct regulatory mechanisms and physiological roles of these isoforms, particularly in calcium handling, myocardial contractility, and adaptive responses to catecholamine stimulation. AC5 plays a key role in cardiac and vascular function, and its inhibition has cardioprotective effects. In contrast, AC6 plays a significant role in vasodilation and is particularly enriched in neonatal tissues, with its overexpression enhancing cardiac repair. The potential of targeting these isoforms for therapeutic intervention, especially in conditions such as heart failure where dysregulated β-adrenergic signaling contributes to disease progression, is also discussed.

Atherosclerosis is a significant underlying cause of cardiovascular disease. It is a chronic, progressive condition marked by the accumulation of plaques composed of lipids, cholesterol, calcium, and cellular debris within the walls of arteries (Wang et al., 2021). Inclisiran is a novel cholesterol-lowering drug with promising pharmacological properties, as described by Zhang et al. The drug utilizes small interfering RNA (siRNA) to silence the expression of the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene, which encodes a protein responsible for degrading low-density lipoprotein (LDL) receptors in the liver. By inhibiting PCSK9 expression, inclisiran increases the availability of LDL receptors on hepatic cells, thereby enhancing the clearance of LDL cholesterol from the bloodstream. This leads to a sustained reduction in LDL cholesterol levels, as demonstrated in clinical trials, thereby reducing the risk of atherosclerosis progression.

Inflammation is a key contributor to the pathogenesis of many cardiovascular diseases such as atherosclerosis. The NOD-like receptor protein 3 (NLRP3) inflammasome is an inflammatory mediator activated in cardiovascular diseases. Upon activation, NLRP3 triggers pyroptosis, a form of programmed cell death characterized by inflammatory cell death and the release of pro-inflammatory cytokines (Zhang et al., 2023). Ding et al. comprehensively discussed the inhibitory effects of exercise on NLRP3 expression and pyroptosis in atherosclerosis, obesity, diabetic cardiomyopathy, myocardial infarction, hypertension, and heart failure. They demonstrated that NLRP3 plays a critical role in the pathogenesis of cardiovascular disease. In addition to these molecular mechanisms, microbial infections have also been implicated in cardiovascular disease. Aramouni et al. suggested that *Helicobacter pylori* infection may contribute to atherogenesis by promoting foam cell formation and triggering a chronic immune response. These findings underscore the multifaceted nature of inflammation in cardiovascular disease, spanning both molecular and infectious contributions.

Traditional Chinese Medicine (TCM) has emerged as a significant source of alternative and complementary therapies, offering bioactive compounds with therapeutic potential against various diseases. Bioactive monomers extracted from TCM, such as geniposide, astragaloside IV, genkwanin, and tanshinone IIA, target non-coding RNAs to alleviate pathological processes associated with atherosclerosis. These non-coding RNAs modulate mechanisms underlying atherosclerosis, for instance inflammation, oxidative stress, adipogenesis, apoptosis, and autophagy (Liu et al.). In addition to their role in atherosclerosis, non-coding RNAs are also implicated in broader cardiovascular diseases. Active monomers such as quercetin, tripterine, notoginsenoside R1, and berberine—also derived from TCM—reduce myocardial fibrosis effects by modulating non-coding RNAs (Wang et al.). These findings emphasize the broad therapeutic potential of targeting non-coding RNAs in the treatment of various cardiovascular conditions.

In the context of cardiovascular disease, Shenfu, a TCM formulation derived from ginseng (*Panax ginseng* C.A. Mey) and aconite (*Aconitum carmichaelii* Debeaux), may have therapeutic benefits. Injecting Shenfu improves cardiac function in patients diagnosed with myocardial infarction, cardiac arrest following resuscitation, and heart failure (Xu et al.). In animal models of cardiovascular disease, it attenuates oxidative stress and inflammation by downregulating the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway. Additionally, it reduces apoptosis and fibrosis by modulating the transforming growth factor-beta (TGF-β)/Smads signaling pathway. Moreover, ginsenoside Rg3, extracted from *P. ginseng*, has shown promising effects in mitigating heart failure by reducing inflammation, oxidative stress, apoptosis, and fibrosis in various animal models of heart disease and mental illness (Shi et al.).

Zhang et al. conducted a comprehensive review on amentoflavone, a flavonoid with pharmacological potential for treating neurological disorders and cardiocerebrovascular diseases by mitigating inflammation, oxidative stress, and reducing lipid levels. They highlight the challenges in improving its pharmacokinetic profile due to its low solubility and poor oral bioavailability. The study by Wang et al. examined the challenges associated with tyrosine kinase inhibitors, a class of tumor-targeted therapies that includes apatinib. These drugs can cause microcirculatory rarefaction that impairs microvascular growth. The authors hypothesized that inhibiting the Notch signaling pathway may mitigate the microvascular changes induced by tyrosine kinase inhibitors.

Heat stroke can cause myocardial injury by disrupting electrolytes, particularly those associated with the sodium-potassium pump (Wang et al., 2019). Xia et al. explored the pathogenesis of heat stroke-induced myocardial injury, highlighting hypercytokinemia as a result of increased inflammation, metabolic abnormalities, and protein dysregulation. These changes contribute to endothelial dysfunction, circulatory shock, and ultimately cardiomyocyte death. The article also discusses several strategies for the management of heat stroke.

In conclusion, this Research Topic presents a series of articles that explore various aspects of cardiovascular health. It provides valuable insights into the molecular mechanisms underlying the pathogenesis of cardiovascular disease along with the protective effects of different cardiovascular drugs. Collectively, these articles serve as a vital resource for researchers, clinicians, and healthcare professionals striving to improve patient outcomes in cardiovascular care.

Author contributions

YK: Writing–original draft, Writing–review and editing. IL: Writing–review and editing. MS: Writing–review and editing.

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EDITED BY
Ismail Laher,
University of British Columbia, Canada

REVIEWED BY
Narasimham L. Parinandi,
The Ohio State University, United States
Wen Wang,
Capital Medical University, China

*CORRESPONDENCE
Ali H. Eid,
ali.eid@qu.ed.u.qa

†These authors have contributed equally to this work

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Infection with *Helicobacter pylori* may predispose to atherosclerosis: role of inflammation and thickening of intima-media of carotid arteries

Karl Aramouni[1†], Roland K. Assaf[1†], Maria Azar[1], Karen Jabbour[1], Abdullah Shaito[2], Amirhossein Sahebkar[3], Assaad A. Eid[1], Manfredi Rizzo[4] and Ali H. Eid[5*]

[1]Faculty of Medicine, American University of Beirut, Beirut, Lebanon, [2]Biomedical Research Center, Department of Biomedical Sciences at College of Health Sciences, College of Medicine, Qatar University, Doha, Qatar, [3]Biotechnology Research Center, Applied Biomedical Research Center, Department of Biotechnology, School of Pharmacy, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran, [4]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, University of Palermo, Palermo, Italy, [5]Department of Basic Medical Sciences, College of Medicine, QU Health, Qatar University, Doha, Qatar

Atherosclerosis is a major instigator of cardiovascular disease (CVD) and a main cause of global morbidity and mortality. The high prevalence of CVD calls for urgent attention to possible preventive measures in order to curb its incidence. Traditional risk factors of atherosclerosis, like age, smoking, diabetes mellitus, dyslipidemia, hypertension and chronic inflammation, are under extensive investigation. However, these only account for around 50% of the etiology of atherosclerosis, mandating a search for different or overlooked risk factors. In this regard, chronic infections, by *Helicobacter pylori* for instance, are a primary candidate. *H. pylori* colonizes the gut and contributes to several gastrointestinal diseases, but, recently, the potential involvement of this bacterium in extra-gastric diseases including CVD has been under the spotlight. Indeed, *H. pylori* infection appears to stimulate foam cell formation as well as chronic immune responses that could upregulate key inflammatory mediators including cytokines, C-reactive protein, and lipoproteins. These factors are involved in the thickening of intima-media of carotid arteries (CIMT), a hallmark of atherosclerosis. Interestingly, *H. pylori* infection was found to increase (CIMT), which along with other evidence, could implicate *H. pylori* in the pathogenesis of atherosclerosis. Nevertheless, the involvement of *H. pylori* in CVD and atherosclerosis remains controversial as several studies report no connection between *H. pylori* and atherosclerosis. This review examines and critically discusses the evidence that argues for a potential role of this bacterium in atherogenesis. However, additional basic and clinical research studies are warranted to convincingly establish the association between *H. pylori* and atherosclerosis.

KEYWORDS

cardiovascular disease, oxidative stress, extra-gastric disease, CagA, vitamin B12 deficiency

Abbreviations: Cag-A, Cytotoxin-Associated Gene; IL-6, Interleukin 6; IL-8, Interleukin 8; TNF-α, Tumor necrosis factor α; ICAM-1, Intercellular adhesion molecule 1; CRP, C-Reactive Protein; NGAL, neutrophil gelatinase-associated lipocalin; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

1 Introduction

Atherosclerosis is precipitated by the pathological formation of plaques within blood vessels. These plaques are instigated by endothelium injury followed by infiltration of immune cells and proliferation of vascular smooth muscle cells. Thickening of the intima and hardening of the vessels then ensue, thus narrowing the vascular lumen which progresses into partial or complete obstruction of blood vessels. Atherosclerosis is a dominant cause and risk factor of cardiovascular disease (CVD), a major instigator of global mortality (Herrington et al., 2016; Libby et al., 2019). Some of the specific risk factors include age, smoking, diabetes mellitus, dyslipidemia, hypertension, and chronic inflammation (Siasos et al., 2014; Spence and Pilote, 2015; Aarabi et al., 2018; Libby et al., 2018; Yao et al., 2019; Lechner et al., 2020). These can account only for around 50% of the incidence of atherosclerosis (Herrington et al., 2016; Libby et al., 2019), making investigation into other risk factors of incidence a hot and rather attractive research area. In recent years, some attention has been given to newly identified potential atherosclerosis risk factors like chronic infections (Pothineni et al., 2017). Possible chronic infectious agents that have been reportedly linked to atherosclerosis include the infamous bacterium *Helicobacter pylori* (Karbasi-Afshar et al., 2015).

H. pylori is a gram-negative, microaerophilic, spiral-shaped bacterium. It is a key microorganism of the human microbiome and a colonizer of the gut of at least half of the world's population (Eusebi et al., 2014; Mentis et al., 2015; Burucoa and Axon, 2017; Leja et al., 2019). In 1994, this bacterium was categorized as a human class I carcinogen by the World Health Organization (Wang, 2014). Not only that, but *H. pylori* infection is the major cause of several gastric diseases including acute and chronic active gastritis, chronic atrophic gastritis, peptic ulcer, gastric adenocarcinoma, and type B low-grade mucosa-associated lymphoid tissue lymphoma (Wang et al., 2014; Wroblewski and Peek, 2016; Chang et al., 2018; de Brito et al., 2019; Sugano, 2019; Ford et al., 2020). Importantly, *H. pylori* infection has been linked to more than 50 extra-gastrointestinal manifestations in a variety of medical specializations such as dermatology, endocrinology, hematology, and cardiology, among others (Campuzano-Maya, 2014; He et al., 2022). In particular, insulin resistance (Polyzos et al., 2011), liver disorders (Waluga et al., 2015), ventilator-associated pneumonia (Dadashi and Hosseinzadeh, 2018), osteoporosis (Mizuno et al., 2015), chronic kidney disease (Hata et al., 2021), hematological manifestations like iron deficiency and vitamin B12 (cobalamin) deficiency, hypertension, and atherosclerosis have been linked to *H. pylori* infection (Tsay and Hsu, 2018; Fang et al., 2019; Franceschi et al., 2019; Mansori et al., 2020a; Mansori et al., 2020b).

Involvement of *H. pylori* in this wide array of diseases may be partly related to the fact that this bacterial infection contributes not only to local inflammation but also to systemic inflammation (Tsay and Hsu, 2018; Fang et al., 2019; Franceschi et al., 2019; Mansori et al., 2020a; Mansori et al., 2020b). In turn, the latter can instigate several extra-gastrointestinal disorders such as metabolic syndrome, diabetes mellitus, insulin resistance, and CVD (Tsay and Hsu, 2018; Fang et al., 2019; Franceschi et al., 2019; Mansori et al., 2020a; Mansori et al., 2020b; Kountouras et al., 2021). Of particular interest, recent studies have found that *H. pylori* may be responsible for the initiation, progression, and complications of atherosclerotic plaque formation. Hence, this review was undertaken to critically examine the evidence concerning *H. pylori* chronic infection as a risk factor of atherosclerosis.

2 Current evidence

2.1 *H. pylori* infection modifies carotid intima-media thickness (CIMT)

One early indication of atherosclerosis is thickening of the carotid artery. In atherosclerosis, this blood vessel narrows, and blood flow becomes compromised thereby increasing the risk of CVD. Indeed, CIMT, which measures the thickness of the inner two layers (the intima and media) of a carotid artery, is an early diagnostic tool of atherosclerosis as this thickening appears even in asymptomatic pre-atherosclerosis patients. Relatedly, several reports support a positive correlation between *H. pylori* infection and an increase of CIMT (Table 1). Indeed, CIMT values were significantly higher in *H. pylori*-infected versus non-infected subjects, and the levels of *H. pylori*-IgG were positively correlated with the increased CIMT measurements (Shan et al., 2018). Similar results were obtained in *H. pylori*-positive men, younger than 50 years of age, who showed a higher incidence of carotid atherosclerosis than non-*H. pylori*-infected counterparts (Zhang et al., 2019). In addition, subjects without carotid atherosclerosis, but with *H. pylori* infection, were found to have higher CIMT values than those free of the infection (Zhang et al., 2019). Importantly, a follow up of subjects with persistent *H. pylori* infection after 5 years revealed a significantly higher incidence of carotid atherosclerosis compared to subjects who were *H. pylori* negative (Zhang et al., 2019). This clearly shows a potentially causative effect of this bacterial infection with atherosclerosis. Indeed, a cross-sectional study shows a positive association between *H. pylori* infection, CIMT and carotid atherosclerosis, independent of classical risk factors (Zhang et al., 2021). In agreement with this, several meta-analyses show that *H. pylori* infection can significantly increase CIMT and lead to subclinical atherosclerosis (Wang et al., 2021b; Shi et al., 2022; Simon et al., 2022).

One of the key interplayers in the potential association between atherosclerosis and *H. pylori* infection is the cytotoxin-associated gene A (CagA). Indeed, studies involving both the right and left coronary artery of patients who underwent an upper GI endoscopy show higher CIMT values among patients infected with a *H. pylori* strain positive for CagA, compared to patients infected with CagA-negative *H. pylori* (Talari et al., 2021). The CagA-positive group also exhibited higher levels of high-sensitivity C-reactive protein (hsCRP), a marker of elevated inflammatory response (Talari et al., 2021). These studies led to the conclusion that CagA+ *H. pylori* strain can induce a systemic inflammatory response that may contribute to the development of atherosclerosis (Talari et al., 2021). Notably, changes in CIMT values were found to be even more prominent when *H. pylori* infection was coupled with certain comorbidities. For instance, CIMT values were highest among subjects with an *H. pylori* infection and alcoholic liver disease (ALD), compared to other groups with only one or neither of the conditions (Bao-Ge et al., 2017). Additionally, the coexistence of *H. pylori* infection and early-stage diabetic kidney disease (DKD) causes a significant increase in CIMT measurements, and potentiates the risk of developing atherosclerosis in type

TABLE 1 Major evidence correlating *H. pylori* infection with atherosclerosis.

Evidence	Findings	References
CIMT	Positive correlation between high CIMT values and *H. pylori* infection	Shan et al. (2018), Zhang et al. (2019), Wang et al. (2021b), Talari et al. (2021), Zhang et al. (2021), Shi et al. (2022), Simon et al. (2022)
	Correlation exacerbated when *H. pylori* infection is coupled with certain comorbidities	Bao-Ge et al. (2017), Feng et al. (2018a), Yu et al. (2019), Choi et al. (2022)
Dyslipidemia	H. *pylori* infected subjects have lower levels of HDL and higher levels of LDL.	Lee et al. (2018), Abdu et al. (2020), Kim et al. (2016), Liang et al. (2021), Choi et al. (2019), Medrek-Socha et al. (2018), Shan et al. (2017)
	H. *pylori* eradication therapy increases HDL level and restores LDL/HDL ratio	Iwai et al. (2019), Park et al. (2021)
Systemic immune response	Chronic inflammation caused by *H. pylori* infection can generate persistent oxidative stress and modify LDL into oxidized-LDL.	Hansson et al. (2006), Sies et al. (2017), Butcher et al. (2017), Gao and Liu (2017), Luo et al. (2017), Krupa et al. (2021)
	Serum Ox-LDL and 8-OHdG levels are higher in T2DM patients with *H. pylori* infection	Nasif et al. (2016)
	Potential association between *H. pylori*, the inflammatory cytokine YKL-40, and atherosclerosis	Cooke et al. (2001), Wu et al. (2004), Martinet et al. (2002), Rathcke and Vestergaard (2006), Xu et al. (2016)
Vitamin B12 deficiency	*H. pylori*-induced atrophic gastritis and reduction in the levels of intrinsic factor protein lead to Vitamin B12 deficiency	Supiano (1987), Kaptan et al. (2000), Carmel et al. (2001), Dierkes et al. (2003), Sipponen et al. (2003), Soyocak et al. (2021)
	H. pylori–induced vitamin B12 deficiency is suggested to play a key role in atherosclerosis via several mechanisms one of which is inhibition of the enzyme methionine synthase leading to high production of homocysteine. Hyperhomocysteinemia is implicated in atherosclerosis via several mechanisms	Zhang et al. (2000), Zhang et al. (2009), Steed and Tyagi (2011), Kozlowski et al. (2012), Zhao et al. (2017), Wu et al. (2019)
CagA	CagA is released systemically from CagA-injected gastric epithelial cells via exosomes. At the vascular endothelium, the virulence factor can stimulate NF-κB and STAT3 in endothelial cells and promote the mounting of an immune reaction at the atherosclerotic plaques by interaction with anti-CagA antibodies	Ansari and Yamaoka (2020), Tegtmeyer et al. (2017), Shimoda et al. (2016), Tahmina et al. (2022), Qiang et al. (2022)
	CagA+ exosomes cause endothelial dysfunction	Guo et al. (2021), Xia et al. (2022)
	When taken up by macrophages in plaques, CagA accelerates foam cells formation	Yang et al. (2019)
	CagA promotes platelets aggregation	Byrne et al. (2003); Riad (2021)

Abbreviations: CIMT, carotid intima-media thickness test; Ox-LDL, oxidized low-density lipoprotein; 8-OHdG, 8-hydroxy-2′-deoxyguanosine, T2DM, Type 2 diabetes mellitus.

2 diabetic patients (Feng et al., 2018a). In confirmation, two recent studies revealed that patients with both *H. pylori* infection and nonalcoholic fatty liver disease (NAFLD) exhibited the highest risk of carotid artery plaque formation and arterial stiffness (Yu et al., 2019; Choi et al., 2022). Taken together, these findings support the notion that *H. pylori* infection is associated with higher CIMT measurements, suggesting a correlation with the eventual incidence of atherosclerosis, in healthy individuals or ones with other co-morbidities.

2.2 *H. pylori* infection precipitates dyslipidemia

Maintaining appropriate blood lipid profiles is critical for the prevention of atherosclerotic plaque buildup. Among serum lipids, cholesterol exhibits a high tendency to accumulate on vascular walls, potentially narrowing the lumen and obstructing blood flow. Interestingly, one of the major mechanisms by which *H. pylori* could precipitate the progression of atherosclerosis is by modifying serum lipid levels and profiles (Table 1) (Vijayvergiya and Vadivelu, 2015).

Current evidence suggests that *H. pylori* is responsible for an impairment in lipid metabolism (Adachi et al., 2018). A chronic infection with *H. pylori* can modify host body lipid distribution by activating pro-inflammatory factors, decreasing lipolysis, and enhancing *de novo* synthesis of fatty acids in the liver (Wang et al., 2022). *H. pylori* can also directly act on the liver to modify body lipid profiles by inducing liver dysfunction and elevating small intestinal mucosal permeability, facilitating the invasion of bacterial endotoxins to the liver through the portal vein, causing hepatic tissue damage (Wang et al., 2022). Importantly, *H. pylori* was reported as an independent risk factor for impaired lipid profiles, manifested as reduced high-density lipoprotein (HDL) and elevated low-density lipoprotein (LDL) levels (Buzás, 2014; Zhao et al., 2019; Hashim et al., 2022; Wang et al., 2022). It is not surprising then that dyslipidemia is prevalent in *H. pylori*-suspected patients where 87.2% of *H. pylori* positive subjects had at least one abnormality in lipid profile (Abdu et al., 2020). This argument is cemented by the finding that *H. pylori*-triggered deterioration in lipid metabolism or dyslipidemia is alleviated when this bacterium is eradicated (Park et al., 2021; Wang et al., 2022).

A recent study aiming to evaluate whether current *H. pylori* infection, detected using a rapid urease test [Campylobacter-like organism test (CLO)], is correlated with subclinical

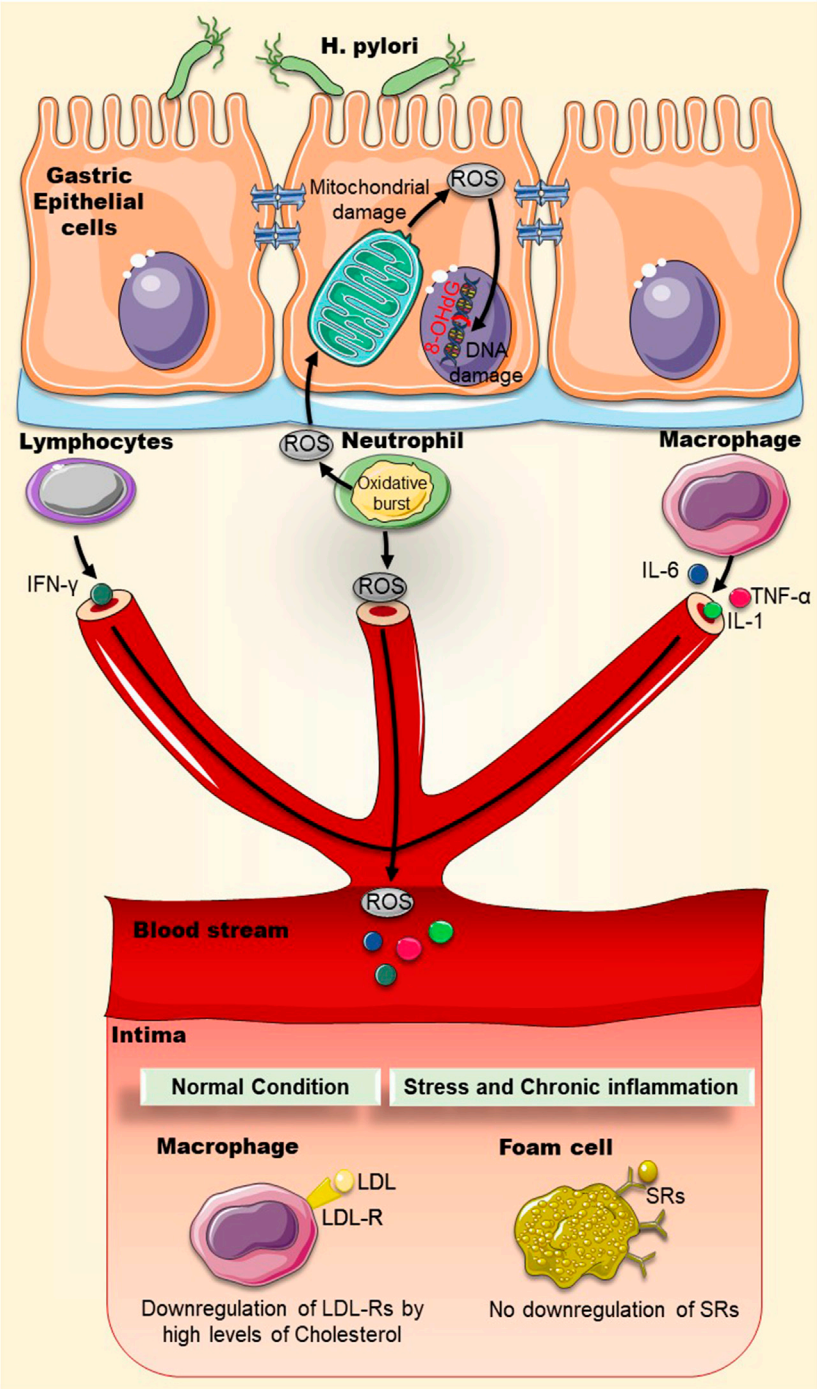

FIGURE 1
Chronic inflammation and oxidative stress may increase the uptake of oxidized-LDL particles by scavenger receptors on macrophages. Chronic *H. pylori* infection can cause local and systemic inflammation that may accelerate atherosclerotic plaque formation. Oxidative stress (ROS) and inflammatory markers (IL-1, IL-6, TNF-α, IFN-γ) generated by immune cells and mitochondrial membrane disruption can damage DNA where deoxyguanosine becomes oxidized into 8-OHdG. LDL particles are modified by oxidative stress into oxidized-LDL (Ox-LDL). Ox-LDL particles do not bind to LDL-Rs but rather bind to scavenger receptors (SRs), present on macrophages. Unlike LDL-Rs, SRs are not downregulated by high levels of cholesterol and accumulate more cholesterol and fats into macrophages, causing the eventual conversion of macrophages into foam cells. Abbreviations: LDL: Low-density lipoprotein; Ox-LDL: Oxidized low-density lipoprotein; LDL-R: low-density lipoprotein receptor; ROS: Reactive oxygen species; SRs: Scavenger receptors; 8-OHdG: 8-hydroxy-2′-deoxyguanosine.

atherosclerosis showed that CLO-positive subjects are more likely to have significant coronary artery stenosis compared to CLO-negative subjects (Lee et al., 2018). The CLO-positive subjects also showed lower levels of HDL-cholesterol and higher mean levels of triglycerides compared to the CLO-negative subjects (Lee et al., 2018). The risk of having a

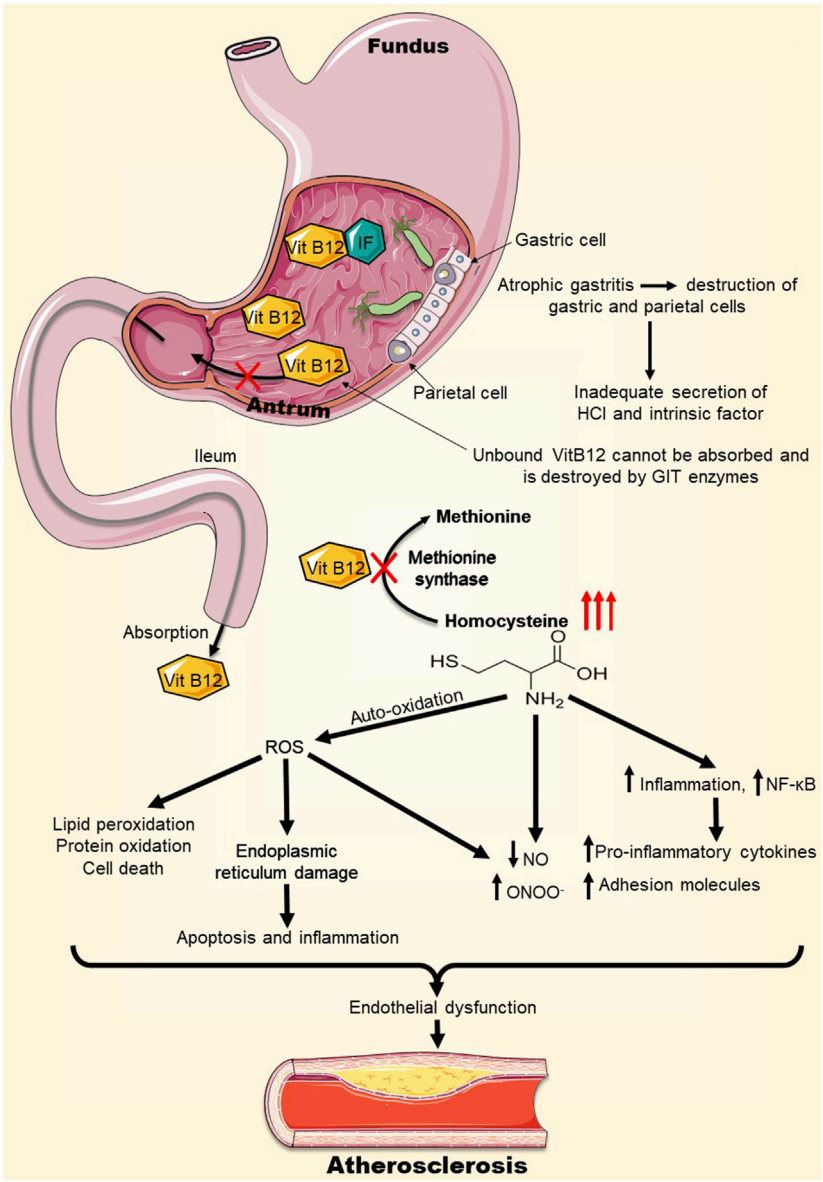

FIGURE 2
H. pylori-induced Vitamin B12 deficiency may precipitate atherosclerosis. *H. pylori* infection can lead to atrophic gastritis and destruction of parietal cells, which secrete intrinsic factor (IF), essential for vitamin B12 absorption. As a result, *H. pylori* infection can cause vitamin B12 deficiency. Vitamin B12 deficiency inhibits the enzyme methionine synthase, escalating homocysteine levels. High levels of homocysteine in the vasculature can lead to endoplasmic reticulum stress, oxidative stress, and inflammation, overall causing endothelial dysfunction which promotes atherosclerosis. Abbreviations: IF, Intrinsic Factor; ROS, Reactive oxygen species; NO, Nitric oxide; $ONOO^-$, Peroxynitrite; NF-kB, Nuclear factor Kappa B.

coronary stenosis was even more prominent after adjusting for age, sex, and other factors that influence coronary artery stenosis, such as systolic blood pressure (BP), fasting glucose, HDL-cholesterol, anti-hypertension/diabetic medications, lipid-lowering agents, and antiplatelet agents (Lee et al., 2018). A recent cross-sectional study showed that *H. pylori*-infected patients exhibit higher LDL, triglycerides and cholesterol levels than control patients' (Nigatie et al., 2022). This is further supported by other findings showing that *H. pylori* seropositivity is a significant risk factor for higher levels of LDL-cholesterol, triglycerides, BMI and lower levels of HDL-cholesterol, further establishing a role for *H. pylori* infection in dyslipidemia (Table 1) (Kim et al., 2016).

In asymptomatic healthy individuals, arterial stiffness, LDL-cholesterol levels, and the prevalence of dyslipidemia were significantly higher in the *H. pylori*-seropositive group compared to the *H. pylori*-seronegative group (Choi et al., 2019). In confirmation, findings from a recent study where participants were divided into three groups (healthy non-*H. pylori* infected, and symptomatic and asymptomatic *H. pylori* infected individuals), indicated that cholesterol levels were significantly higher in the symptomatic group than the asymptomatic and healthy groups (Medrek-Socha et al., 2018). Relevantly, both of the infected groups

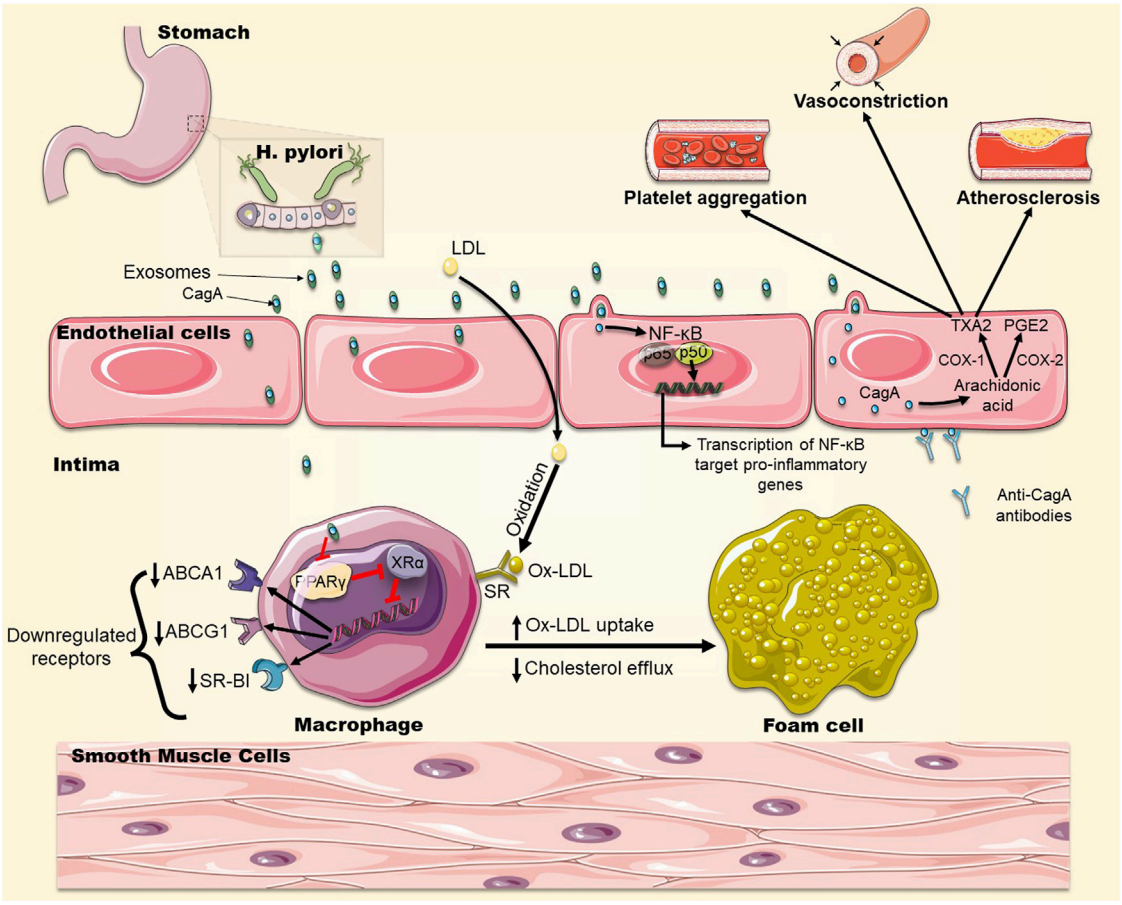

FIGURE 3
The role of CagA in foam cell formation. CagA is released in exosomes from *H. pylori*-infected gastric cells and becomes engulfed by macrophages in the intima of arteries. CagA downregulates the expression of transcription factors PPARγ and LXRα, which control the expression of the cholesterol efflux transporters ABCA1, ABCG1 and SR-BI on the surface of macrophages leading to cholesterol accumulation in macrophages and promoting foam cell formation. In addition, OxLDL infiltrates macrophages via SRs which are not downregulated by cholesterol, exacerbating foam cell formation. CagA also stimulates NF-kB signaling leading to the upregulation of expression of pro-inflammatory genes. CagA also elevates the levels of COX-1 and COX-2, leading to a higher production of TXA2 and PGE2, promoting platelets aggregation and vasoconstriction. CagA can be expressed on cells of the atherosclerotic plaque, to which anti-CagA antibodies will bind, exacerbating immune reactions.

showed higher LDL and lower HDL concentrations than the healthy group (Medrek-Socha et al., 2018). Other supporting data include the finding that subjects infected by *H. pylori*, specifically males between 55 and 74 years of age, had significantly higher levels of LDL-cholesterol (Shan et al., 2017). A recent observational study in chronic gastritis patients showed that *H. pylori* eradication therapy causes a significant increase in HDL levels and a significant decrease of LDL/HDL ratio, a measure of the risk of atherosclerosis (Iwai et al., 2019). Taken together, it is becoming increasingly evident that infection with *H. pylori* is associated with dyslipidemia, a key contributing factor to atherosclerotic plaque formation.

2.3 *H. pylori* infection can induce systemic immune responses

Key parameters implicated in systemic inflammation are adhesion molecules and pro-inflammatory cytokines. In this context, it has been reported that *H. pylori* can induce upregulation of adhesion molecules on gastric epithelium as well as promote the release of several cytokines like IL-1, IL-6 and TNF -α, which then activate leukocytes and precipitate systemic inflammation (Vijayvergiya and Vadivelu, 2015). This *H. pylori*-induced chronic inflammation can precipitate persistent oxidative stress, with various noxious effects such as DNA damage, mitochondrial membrane damage and pro-inflammatory immune responses (Butcher et al., 2017; Sies et al., 2017). This contributes to a wide range of extra-gastrointestinal tract abnormalities, including atherosclerosis.

Under normal oxidative conditions, LDL particles are taken up by macrophages via receptor-mediated endocytosis by the LDL-Receptors (LDL-Rs). LDL-Rs can be downregulated by elevated levels of cholesterol, limiting the uptake of fat. However, LDL particles are extremely sensitive to oxidative damage and can be modified into oxidized-LDL (Ox-LDL) during chronic *H. pylori* infection, for instance. Ox-LDL are

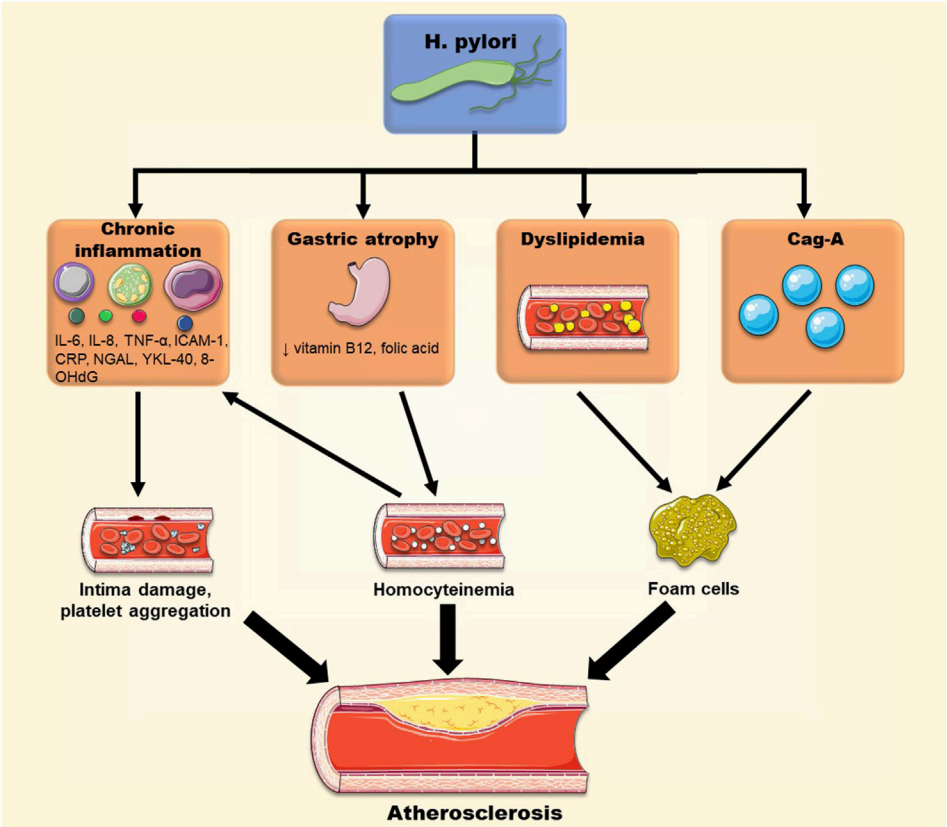

FIGURE 4
Different pathways through which *H. pylori* may promote atherosclerotic plaque formation. *H. pylori* infection can have systemic effects and cause chronic inflammation, dyslipidemia, Vitamin B12 deficiency and homocysteinemia, and promote foam cell formation, contributing to the progression of atherosclerosis.

atherogenic because they do not bind to LDL-Rs but have the capacity to bind and activate a group of receptors collectively known as scavenger receptors (SRs). These SRs are present on macrophages, but unlike LDL-Rs they are not downregulated by high levels of cholesterol, and can keep on accumulating cholesterol and fats, eventually causing the conversion of macrophages into foam cells, a hallmark of atherosclerosis (Figure 1) (Gao and Liu, 2017; Luo et al., 2017). When lipoproteins accumulate in the intima, the endothelium becomes poised to secrete a repertoire of chemokines and display several leukocyte adhesion molecules, favoring the recruitment of immune cells. An immune response is then mounted, leading to the accumulation of pro-inflammatory cytokines, proteases, and vasoactive molecules. This local inflammation can exacerbate plaque growth in blood vessels, furthering their obstruction (Hansson et al., 2006).

Ox-LDL levels were also found to be higher in serum of *H. pylori*–infected type II diabetes mellitus (T2DM) patients than in serum of diabetic patients without *H. pylori* infection or non-diabetics (Table 1) (Nasif et al., 2016). More, the role of *H. pylori* infection in accelerating foam cell formation in animals with high-fat diet has also been reported (Krupa et al., 2021). In these animals, a dramatic increase in systemic inflammatory markers fueling a pro-atherogenic endothelial cell environment was noted. Infiltration of inflammatory cells, elevation of oxidative stress, formation of foam cells, and presence of pro-atherogenic molecules like 7-ketocholesterol and aldehydes were prominently evident (Krupa et al., 2021).

Oxidative damage also impacts both nuclear and mitochondrial DNA. For instance, deoxyguanosine becomes oxidized into 8-hydroxy-2′-deoxyguanosine (8-OHdG) (Cooke et al., 2001). Indeed, this oxidized DNA molecule is often used as a marker of oxidative damage. Moreover, it is reportedly found-in rather excessive amounts-in atherosclerotic plaques (Wu et al., 2004), inside macrophages, smooth muscle cells, and endothelial cells, demonstrating strong oxidative DNA damage and repair (Martinet et al., 2002). Likewise, levels of 8-OHdG and serum Ox-LDL levels in T2DM patients appear to be directly associated with *H. pylori* infection (Nasif et al., 2016). This lends support to the notion that *H. pylori* infection contributes to the pathogenesis of atherosclerosis by virtue of increasing serum Ox-LDL and 8-OHdG (Figure 1).

An interesting relation between vascular dementia, inflammation and atherosclerosis is emerging. Indeed, *H. pylori*-positive patients with vascular dementia (VD) had greater CIMT values and higher levels of YKL-40 cytokine [a biomarker of inflammation (Rathcke and Vestergaard, 2006)] than *H. pylori*-negative VD patients (Xu et al., 2016). Additionally, CIMT was

positively correlated with serum levels of YKL-40 cytokine independent of traditional atherosclerotic risk factors (Xu et al., 2016). This finding suggests a potential association between *H. pylori*, YKL-40 cytokines and atherosclerosis, warranting further investigation (Xu et al., 2016). Overall, *H. pylori* infection-induced inflammation may be able to precipitate the development of atherosclerosis as summarized in Table 1.

2.4 *H. pylori* infection can cause vitamin B12 deficiency

Atrophic gastritis is chronic inflammation of the gastric mucosa that can result in stomach atrophy along with low or absent gastric acid secretion and inadequate production of intrinsic factor (IF). This factor is a protein that binds and facilitates the transit of vitamin B12 (cobalamin) through the small intestine to be absorbed into the bloodstream (Hall and Appelman, 2019). In the absence of gastric secretions or IF, the absorption of food-bound vitamin B12 is impaired (Neumann et al., 2013). As a result, people suffering from chronic atrophic gastritis have been documented to suffer from cobalamin deficiency (Allen, 2008). Of particular interest to this review, an untreated *H. pylori* infection can eventually cause atrophic gastritis, implicating *H. pylori* in vitamin B12 deficiency (Supiano, 1987). This causality was investigated in several studies, with the conclusion that there is a probable association between cobalamin deficiency and *H. pylori* infection (Kaptan et al., 2000; Carmel et al., 2001; Dierkes et al., 2003; Sipponen et al., 2003; Raut and Chandel, 2014; Civan, 2020; Soyocak et al., 2021). These findings have potential implications to atherosclerosis because vitamin B12 malabsorption can promote the progression of atherosclerosis (Zhao et al., 2017). In fact, cobalamin is a cofactor for the enzyme methionine synthase catalyzing the conversion of homocysteine to methionine (Kozlowski et al., 2012). In the case of vitamin B12 deficiency, methionine synthase is non-functional, escalating homocysteine levels (Sipponen et al., 2003). A recent study comparing homocysteine levels of *H. pylori* positive subjects to those of *H. pylori* negative individuals found that homocysteine levels were significantly higher in the infected subjects (Huang et al., 2022).

Hyperhomocysteinemia (HHcy) is suggested to play a key role in atherosclerosis via several mechanisms (Zhao et al., 2017). The auto-oxidation of homocysteine is a source of reactive oxygen species (ROS), which at high levels in the vasculature can cause lipid peroxidation, protein oxidation, and even cell death, eventually leading to vascular injuries (Steed and Tyagi, 2011). HHcy by itself as well as HHcy-generated ROS can induce peroxynitrite ($ONOO^-$) formation, effectively reducing nitric oxide (NO) bioavailability in the vasculature. Generation of $ONOO^-$ and the decrease of NO levels can cause dysfunction of the vascular endothelium, instigating atherosclerosis and other diseases of the vasculature (Zhang et al., 2000). HHcy-induced oxidative stress can as well affect the endoplasmic reticulum of endothelial cells, thereby inducing apoptosis and inflammation, and dysregulation of lipid metabolism (Wu et al., 2019). Furthermore, elevated levels of homocysteine can induce the activation of nuclear factor kappa B (NF-κB) transcription factor which activates the expression of different cytokines, chemokines, and leukocyte adhesion molecules; important mediators of vascular inflammation, pro-thrombotic state, and atherogenesis (Figure 2) (Cattaneo, 1999; Zhang et al., 2009; Riad, 2021). Taken together, it is becoming more evident that *H. pylori*-induced vitamin B12 malabsorption can lead to elevated levels of homocysteine, which may then promote the development of atherosclerosis by various mechanisms (Figure 2).

2.5 The role of CagA in atherosclerosis

CagA, a virulence factor protein expressed by certain strains of *H. pylori,* is responsible for the development of gastric diseases and is suspected to be involved in extra-gastric diseases (Ansari and Yamaoka, 2020). During infection, *H. pylori* injects this cytotoxin into host gastric cells, through a Type IV secretion system, where CagA can modify host cell signaling pathways (Tegtmeyer et al., 2017). *H. pylori*-infected gastric epithelial cells were found to release extracellular vesicles (exosomes) containing CagA. These exosomes can be delivered, through the bloodstream, to vascular endothelia distant from the primary site of infection (Table 1) (Shimoda et al., 2016). This finding may be one way to explain the systemic clinical effects thought to be mediated by *H. pylori*, including atherosclerosis. Indeed, these circulating CagA-containing exosomes may adhere to distant endothelial monolayers and deliver the CagA protein into endothelial cells, where it can stimulate NF-κB signaling. This signaling may lead to a chronic inflammatory condition, endothelial cell dysfunction, and consequently plaque formation. Similarly, Wang *et al.* reported that extracellular vesicle (outer membrane vesicles) derived from $CagA^+$ *H.pylori*-infected cells accelerated atherosclerotic plaque formation in $ApoE^{-/-}$ mice via NF-κB signaling (Figure 3) (Wang et al., 2021a). Exosomes-delivered CagA to endothelial cells were also found to activate the pro-inflammatory transcription factor STAT3 (Tahmina et al., 2022). In another mechanism, CagA-containing exosomes may directly fuse with cells of the atherosclerotic plaques, exposing CagA on the plaque surface to be recognized by anti-CagA antibodies, leading to immune reactions at the atherosclerotic plaque (Figure 3) (Shimoda et al., 2016).

PMSS1 is a CagA-positive *H. pylori* strain which can secrete the CagA virulence factor into host cells. PMSS1 *H. pylori*-infected mice have shown accelerated growth of atherosclerotic plaques that was dependent on dietary risk factors and genetic susceptibility (Yang et al., 2019). Immunohistochemical analysis of the vasculature of these mice revealed that the atherosclerotic plaques contained macrophage-derived foam cells. These cells significantly accumulate neutral lipid in the presence of PMSS1 infection, suggesting that the bacteria may help in the formation of foam cells (Yang et al., 2019).

This notion was reinforced by other *in vitro* and *in vivo* studies. Exosomes derived from *H. pylori*-infected gastric epithelial cells (Hp-GEC-EVs), were able to induce conversion of macrophages into foam cells when taken up by macrophages. Interestingly, CagA present in Hp-GEC-EVs would end up in the atherosclerotic plaques leading to exacerbation of the obstructive inflammatory process (Yang et al., 2019). It was also found that CagA mediated the formation of foam cells through downregulation of the expression of transcription factors PPARγ and LXRα, which control the expression of the cholesterol efflux transporters ABCA1, ABCG1 and SR-BI on the surface of macrophages, leading to cholesterol accumulation in macrophages and promoting foam cell

formation (Figure 3) (Yang et al., 2019). A recent study comparing the virulence of CagA$^+$ *H. pylori* to CagA$^-$ *H. pylori* in mice found that the former strain colonized gastric mucosa more effectively, causing endothelial dysfunction and enhancing atherosclerosis through ROS production, which was induced by CagA$^+$ exosomes (Xia et al., 2022). Another study demonstrated that *H. pylori* infection was an independent risk factor for intracranial atherosclerosis, especially in women younger than 60 years of age, and that CagA$^+$ exosomes significantly limited brain endothelial functioning *in vitro* (Guo et al., 2021). As well, a meta-analysis showed that *H. pylori* infection can significantly increase CIMT, especially in CagA$^+$ individuals (Shi et al., 2022).

CagA-positive *H. pylori* strains can also promote platelet aggregation. Indeed, CagA is suspected to increase cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) production in the vascular endothelium resulting in higher thromboxane (TXA2) and prostaglandins levels, both of which are known to modify platelet aggregation and contribute to atherosclerosis (Figure 3) (Byrne et al., 2003; Riad, 2021).

Hence, CagA$^+$ *H. pylori* infection may accelerate atherosclerotic plaque formation via several CagA-dependent mechanisms: CagA packaged in exosomes can modify macrophage intracellular cholesterol levels by increasing the import of cholesterol or decreasing cholesterol efflux which facilitates foam cell formation. Otherwise, CagA can promote the development of atherosclerotic plaques via the stimulation of NF-κB, platelet aggregation, and the interaction with anti-CagA antibodies (Figure 3).

3 The counter-evidence

Despite the multitude of studies demonstrating a positive association between *H. pylori* infection and atherosclerosis, some studies report otherwise (Ahmadnia et al., 2013; Jukic et al., 2017; Feng et al., 2018b; Wernly et al., 2022). A recent cross-sectional study aiming to examine the relationship between *H. pylori* infection and the severity of coronary atherosclerosis included patients with coronary artery disease (CAD) and who underwent coronary artery bypass grafting surgery. Coronary angiograms were scored by quantitative assessment based on three angiographic parameters: the vessel score, Gensini score, and angiographic severity score. The results showed that hypertension, systolic pressure, diastolic pressure, and total cholesterol values were higher in *H. pylori*-positive subjects compared to *H. pylori*-negative subjects. HDL-cholesterol values, on the other hand, were markedly lower in the *H. pylori*-positive than in the negative subjects. However, no significant differences were found in vessel score, Gensini score and angiographic severity score, or in the use of medications that affect atherogenesis, concluding that the pathogenesis of stable chronic CAD is mainly caused by traditional risk factors rather than the influence of *H. pylori* infection (Jukic et al., 2017). Furthermore, a cross-sectional investigating the correlation between the average of CIMT values and *H. pylori* infection did not report significant differences in CIMT measurements between *H. pylori* positive and negative subjects (Feng et al., 2018b). However, traditional atherosclerosis risk factors (like gender, BMI, waistline, lipid profiles) did correlate with significant differences in CIMT scores suggesting no correlation between *H. pylori* infection and early atherosclerosis (Feng et al., 2018b). Moreover, the presence of *H. pylori* in atherosclerotic plaques was investigated in the iliac arteries of 25 patients with end stage renal disease (ESRD) who were undergoing kidney transplantation. Although atherosclerotic plaques were present in 21 patients (84%), *H. pylori* was not detected by polymerase chain reaction and no significant relationship between atherosclerosis and gastric *H. pylori* infection could be established (Ahmadnia et al., 2013). Moreover, despite reporting an independent risk between *H. pylori* infection and CVD, the difference did not translate into CVD mortality between patients with and without the bacteria in a cohort (Wernly et al., 2022). Hence, conflicting data exist regarding the relationship between *H. pylori* and atherosclerosis and further investigations are needed in order to elucidate this relationship.

4 Conclusion

The extra-gastrointestinal manifestations of *H. pylori* infection have linked this pathogen to the progression of atherosclerosis. In addition, the bacteria may account for the development of atherosclerotic plaques through several mechanisms including dyslipidemia, systemic inflammation, oxidative stress and acceleration of foam cell formation. When different strains of the bacteria were compared, *H. pylori* strains positive for CagA showed a higher correlation with the incidence and pathogenesis of atherosclerosis, through various mechanisms involving exosomes, for example, (Figure 4). As such, interventions to eradicate *H. pylori* may help in the reduction of the prevalence of atherosclerosis. Paradoxically, some studies could not establish a role for *H. pylori* in atherosclerosis (Ahmadnia et al., 2013; Jukic et al., 2017; Feng et al., 2018b). The contradiction may be related to the presence of confounding variables, such as the methods used for diagnosis of *H. pylori* diagnostic (serology vs. rapid urease test), selection bias of geographic regions, or the study of different *H. pylori* strains, like CagA-positive or CagA-negative strains. In conclusion, *H. pylori* may exacerbate atherosclerosis, but further supporting evidence is warranted to place it alongside the traditional risk factors of atherosclerosis.

Author contributions

KA: Writing–original draft. RA: Writing–original draft. MA: Writing–original draft. KJ: Writing–original draft. AbS: Writing: review and editing. AmS: Writing: review and editing. AAE: Writing: review and editing. MR: Writing: review and editing. AHE: Conceptualization, Funding acquisition, Project administration, Supervision, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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EDITED BY
Ismail Laher,
University of British Columbia, Canada

REVIEWED BY
Sandhiya Selvarajan,
Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), India
Wei Jie,
Hainan Medical University, China
Željko Reiner,
University Hospital Centre Zagreb, Croatia

*CORRESPONDENCE
Liu Yang,
ciwujia@foxmail.com
Fan Liu,
liufanzyy@163.com

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Inclisiran: a new generation of lipid-lowering siRNA therapeutic

Yanzhen Zhang[1], Huaigang Chen[2,3], Lang Hong[3], Hong Wang[3], Bin Li[3], Mengyin Zhang[4], Jiamei Li[5], Liu Yang[3]* and Fan Liu[6]*

[1]Department of Rheumatology, Shunyi Hospital, Beijing Traditional Chinese Medicine Hospital, Beijing, China, [2]Medical College of Nanchang University, Nanchang, China, [3]Department of Cardiology, Jiangxi Provincial People's Hospital, The First Affiliated Hospital of Nanchang Medical College, Nanchang, China, [4]Pharmacy Department, Jiangxi Provincial People's Hospital, The First Affiliated Hospital of Nanchang Medical College, Nanchang, China, [5]Laboratory Department, Jiangxi Provincial People's Hospital, The First Affiliated Hospital of Nanchang Medical College, Nanchang, China, [6]Department of Hematology Jiangxi Hospital of Traditional Chinese Medicine, Nanchang, China

Atherosclerotic heart disease (AHD) is a major cause of morbidity and mortality worldwide. Lowering low-density lipoprotein cholesterol (LDL-C) levels is a key strategy to prevent and treat AHD. Inclisiran is a novel siRNA drug that targets proprotein convertase subtilisin/kexin type 9 (PCSK9) gene expression and reduces LDL-C levels with only two or three injections per year. This review summarizes the mechanism, efficacy, safety, and applications of Inclisiran in various populations and settings, based on recent literature. It also compares Inclisiran with other lipid-lowering drugs, especially other PCSK9 inhibitors. We conclude that Inclisiran is a promising lipid-lowering agent that can provide convenience and effectiveness for patients with high cardiovascular risk. However, some challenges and limitations remain for Inclisiran, such as its long-term safety and efficacy, its cost-effectiveness and accessibility, and its interactions and synergies with other drugs. These issues need further investigation and evaluation in future studies.

KEYWORDS

lipid-lowering therapies, cardiovascular risk, PCSK9, Inclisiran, siRNA

1 Introduction

Atherosclerotic heart disease (AHD) remains a global public health problem despite of significant advances in its prevention and treatment (Dong et al., 2022). It is caused by the accumulation of cholesterol and other substances in the arterial walls, which led to narrowing and hardening of the arteries and reduce blood flow to the heart and other organs. This can result in chest pain, heart attack, stroke or death. A positive correlation between circulating low-density lipoprotein cholesterol (LDL-C) levels and coronary heart disease has been demonstrated by numerous epidemiological and clinical studies (Mortensen and Nordestgaard, 2020). LDL-C is a kind of cholesterol that can damage the arteries and increase the risk of atherosclerosis (Ference et al., 2013; Liu et al., 2021). LDL-C is the main carrier of cholesterol in the blood and can be taken up by the arterial wall cells via LDL receptors, contributing to plaque formation and inflammation. This process can lead to atherosclerosis and cardiovascular disease (Robinson et al., 2018). In China, the percentage of adults with dyslipidemia, which is an abnormal level of lipids in the blood, is as high as 33.8%, while the percentage of those with increased LDL-C level, which is a major risk factor for coronary artery disease, is 4.0%. These statistics indicate a high prevalence and burden of lipid metabolic disorders in China (Lu et al., 2021). How to effectively reduce LDL-C is one of the research questions to be answered.

In recent years, PCSK9 has gained much attention as a star target for reducing LDL-C levels in patients (German and Shapiro, 2020). PCSK9 is a protein that regulates the degradation of LDL receptors on the surface of liver cells, thereby affecting the clearance of LDL-C from the blood. PCSK9 binds to LDL receptors and promotes their degradation in lysosomes, thus reducing the number of LDL receptors available for clearing LDL-C from the blood. Therefore, inhibiting PCSK9 can increase the expression of LDL receptors and lower LDL-C levels (Tibolla et al., 2011). At present, the drug research and development targeting PCSK9 is mainly focused on preventing the binding of PCSK9 to the low-density lipoprotein (LDL) receptor. There are two main types of drugs that can achieve this goal: monoclonal antibodies and small interfering RNAs (siRNA). Monoclonal antibodies are proteins that can bind to PCSK9 and block its interaction with LDL receptors (Stroes et al., 2014) SiRNA are short nucleic acids that can silence the expression of PCSK9 gene in liver cells (Reyes-Soffer et al., 2017), inhibiting the expression and secretion of PCSK9 (Fitzgerald et al., 2017).

Among these drugs, monoclonal antibodies that block the binding of PCSK9 to LDL receptors are now available in China. The lipid-lowering effect of this drug is very effective, require monthly or every biweekly injection. However, the shortcoming of low compliance and the high cost of preparation of monoclonal antibody preparations are still the major obstacles to the development of these drugs (Kaddoura et al., 2020). This drug refers to the monoclonal antibodies that target PCSK9, such as evolocumab and alirocumab. These drugs can significantly reduce LDL-C levels and cardiovascular events in patients with dyslipidemia (Stroes et al., 2014; Robinson et al., 2015; Sabatine et al., 2017; Diaz et al., 2021). In the meantime, Alirocumab can reduced the risk of any stroke and ischemic stroke without increasing hemorrhagic stroke (Jukema et al., 2019). However, they also have some drawbacks, such as low patient adherence due to frequent injections, high production cost and limited accessibility. Therefore, new lipid-lowering drugs are needed urgently.

Recent studies have shown that PCSK9 is a key protein that regulates low density lipoprotein receptor (LDL-R) expression in hepatocytes, and is positively correlated with LDL-C levels (Tibolla et al., 2011). Targeting PCSK9 has brought new opportunities for lipid-lowering therapies. However, current treatments still face challenges such as poor medication adherence (Jahangir et al., 2021). However, as an emerging therapeutic approach, the long-term safety, cardiovascular risk reduction efficacy, and applicability across diverse populations of siRNA therapeutics need to be verified through more studies (Kosmas et al., 2018).

Inclisiran (trade name Leqvio ®) is a lipid-lowering novel drug developed by Novartis Group. It is the first siRNA drug approved for the treatment of hypercholesterolemia or mixed dyslipidemia, as well as ASCVD or heterozygous familial hypercholesterolemia (HeFH) patients who need further lowering of LDL-C levels. It is a short-chain, synthetic small interfering ribonucleic acid (siRNA) (Khvorova, 2017). SiRNAs are types of double-stranded RNAs that can induce gene silencing by degrading the complementary mRNAs in a sequence-specific manner. However, some issues and challenges remain for Inclisiran, such as its effect on type 2 diabetes, its long-term outcomes, its affordability and availability, and its combination with other drugs. Moreover, the molecular mechanisms and pathways of Inclisiran-mediated LDL-C reduction and cardiovascular protection need to be clarified, and the development of novel siRNA drugs for lipid metabolism or cardiovascular disease may offer new opportunities for personalized and precision medicine. Considering the important value of Inclisiran as a novel siRNA drug in lipid-lowering therapy, we reviewed the recent literature on this drug, and outlined its mechanism of action, pharmacodynamics, safety evaluation, application in China and future application, etc., to provide a comprehensive overview of the advantages and existing problems of Inclisiran, and to offer reference for its position in lipid-lowering therapy.

2 Methods

2.1 Literature search

A literature search was conducted using PubMed, EMBASE, and SinoMed databases. Search terms pre-defined in titles, abstracts, and keywords were used to identify pertinent studies. The retrieval period spanned from the inception of the databases up to March 2023.

3 Inclisiran delivery and mechanism of action

3.1 Mechanism of action

Inclisiran specifically binds to n-acetylgalactosamine (GalNAc) and the asialoglycoprotein receptor (ASGPR) on liver cell membranes (Kosmas et al., 2018). GalNAc is a sugar molecule that enhances the affinity and specificity of Inclisiran for ASGPR, which is a receptor that mediates the endocytosis of glycoproteins in liver cells. After entering the hepatocytes, it binds to the RNA-induced silencing complex (RISC). RISC is a protein complex that recognizes and cleaves the target mRNA based on the guide strand of siRNA. At the same time, it binds to the mRNA who encoding the PCSK9 protein mediated by the antisense chain, which leads to a decrease in PCSK9 protein production. PCSK9 is a protein that regulates the expression of LDL receptors on the surface of liver cells. PCSK9 binds to LDL receptors and promotes their degradation in lysosomes, thus reducing the number of LDL receptors available for clearing LDL-C from the blood. Therefore, by reducing PCSK9 production, Inclisiran can enhance LDL receptor expression and lower LDL-C levels. To illustrate the mechanism of action of Inclisiran, we present a schematic diagram in Figure 1.

3.2 Inclisiran efficacy

After a single dose of Inclisiran, the level of LDL-C was decreased by about 50% and maintained for up to 6 months (Barale et al., 2021; Miname et al., 2021). It is noteworthy that the silencing complex remains active after mRNA degradation occurs. Therefore, the lipid-lowering effect of Inclisiran is effective in the long term.

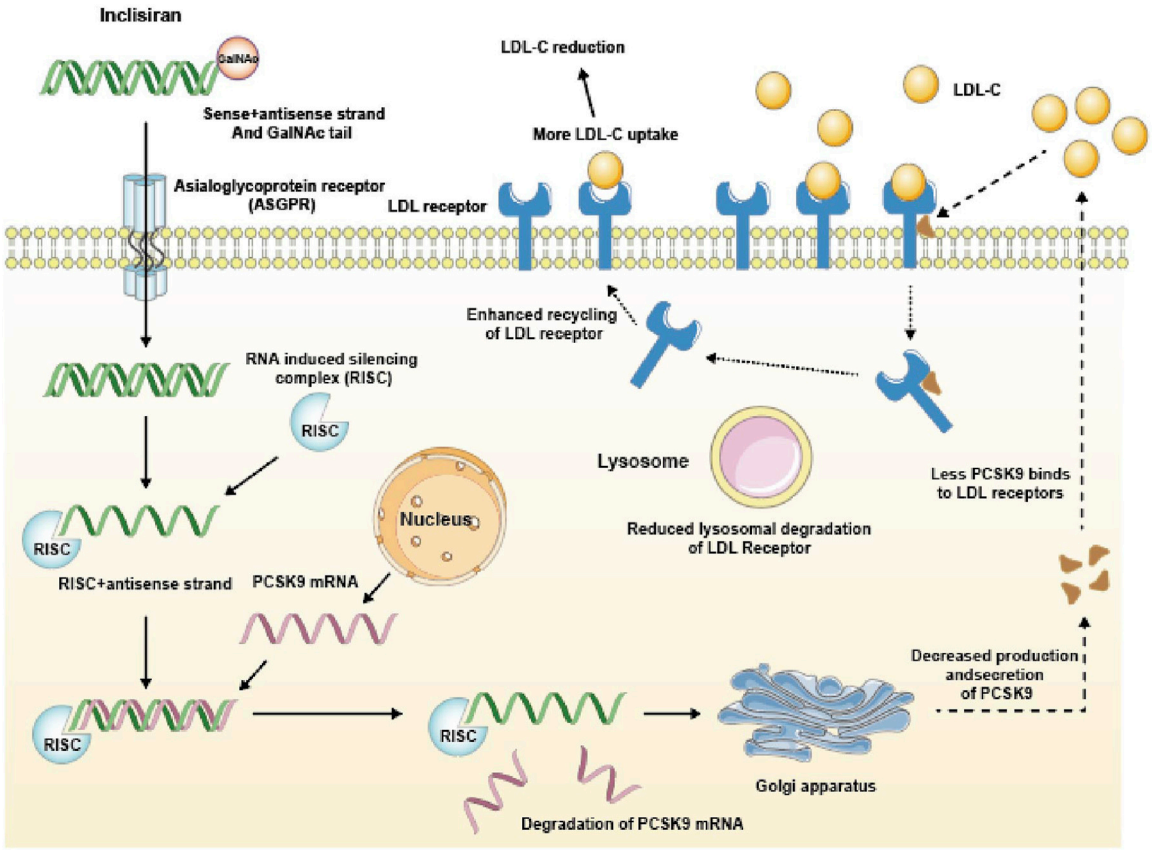

FIGURE 1
Mechanism of action of Inclisiran. GalNAc tail, N-acetylgalactosamine tail; RISC, RNA-induced silencing complex; PCSK9, Proprotein convertase subtilisin/kexin type 9.

4 Clinical efficacy, safety evaluation and exploration and application in China for different types of hypercholesterolemia and cardiovascular disease patients

4.1 Clinical trials of inclisiran for hypercholesterolemia

Inclisiran is involved in five global studies: ORION 4, 9, 10, 11, 18. These studies are phase III, double-blind, randomized, placebo-controlled trials that aim to evaluate the efficacy and safety of Inclisiran in patients with different types of hypercholesterolemia.

4.1.1 ORION 9: inclisiran for heterozygous familial hypercholesterolemia

ORION 9 enrolled patients with heterozygous familial hypercholesterolemia (HeFH), a genetic disorder that causes high LDL-C levels and increased risk of cardiovascular disease. The trial tested the hypothesis that Inclisiran, an innovative siRNA agent that silences PCSK9 gene expression in the liver and increases LDL receptor availability, would reduce LDL-C levels more than placebo in patients who were on maximally tolerated statin and ezetimibe.

4.1.2 ORION 10 and 11: inclisiran for atherosclerotic cardiovascular disease or risk equivalents

ORION 10 and 11 enrolled patients with atherosclerotic cardiovascular disease (ASCVD) or ASCVD risk equivalents, such as diabetes mellitus, chronic kidney disease or peripheral artery disease (Ray et al., 2020). These are conditions that can damage the blood vessels and impair the blood flow to the heart and other organs. The patients enrolled in the study received at least 30 days of treatment with a statin at the maximum tolerable dose. Statins are drugs that can lower LDL-C levels by inhibiting the enzyme that produces cholesterol. The dose of statins was constant throughout the study with or without ezetimibe. Ezetimibe is a cholesterol absorption inhibitor that can lower LDL-C levels by blocking the uptake of cholesterol from the intestine. Enrolled patients were randomized to the Inclisiran group or the placebo group. On the basis of the maximum tolerable dose of statin ± ezetimibe, Inclisiran 300 mg or placebo was given subcutaneously at D0, D90, D270, and D450. The primary endpoint of the studies was the percentage change in LDL-C levels from baseline to day 510. The secondary endpoints included the absolute change in LDL-C levels from baseline to day 510, the time-adjusted percentage change in LDL-C levels from day 90 to day 540, and the safety and tolerability of Inclisiran. The Inclisiran group showed a 51% decrease in LDL-C levels,

TABLE 1 Characteristics and outcomes of the clinical trials of Inclisiran for hypercholesterolemia.

Name	Population	Inclusion criteria	Exclusion criteria	Trial design	Follow-up time	Primary endpoint	Secondary endpoint
ORION-9	Patients with HeFH	18–80 years old, genetic or clinical diagnosis of HeFH, LDL-C ≥ 2.6 mmol/L, maximum tolerated dose of statin ± other lipid-lowering drugs ≥30 days	Pregnant or lactating women, liver dysfunction, other serious diseases or conditions unsuitable for trial participation	Double-blind, randomized, placebo-controlled, multicenter, phase III trial, randomization ratio of 1:1	Observation and measurement on day 0, day 90, day 180, day 270, day 360, day 450 and day 540, for a total of 18 months	Percentage change in LDL-C level from baseline to day 510	Absolute change in LDL-C level from baseline to day 510; time-adjusted percentage change in LDL-C level from day 90 to day 540; safety and tolerability of Inclisiran
ORION-10	Patients with ASCVD or risk equivalent (US)	18–85 years old, with ASCVD or risk equivalent (such as diabetes, chronic kidney disease or peripheral artery disease), LDL-C ≥ 1.8 mmol/L (70 mg/dL), maximum tolerated dose of statin ± other lipid-lowering drugs ≥30 days	Pregnant or lactating women, liver dysfunction, other serious diseases or conditions unsuitable for trial participation	Double-blind, randomized, placebo-controlled, multicenter, phase III trial, randomization ratio of 1:1	Observation and measurement on day 0, day 90, day 180, day 270, day 360, day 450 and day 540, for a total of 18 months	Percentage change in LDL-C level from baseline to day 510	Absolute change in LDL-C level from baseline to day 510; time-adjusted percentage change in LDL-C level from day 90 to day 540; safety and tolerability of Inclisiran
ORION-11	Patients with ASCVD or risk equivalent (Europe)	18–85 years old, with ASCVD or risk equivalent (such as diabetes, chronic kidney disease or peripheral artery disease), LDL-C ≥ 1.8 mmol/L (70 mg/dL), maximum tolerated dose of statin ± other lipid-lowering drugs ≥30 days	Pregnant or lactating women, liver dysfunction, other serious diseases or conditions unsuitable for trial participation	Double-blind, randomized, placebo-controlled, multicenter, phase III trial, randomization ratio of 1:1	Observation and measurement on day 0, day 90, day 180, day 270, day 360, day 450 and day 540, for a total of 18 months	Percentage change in LDL-C level from baseline to day 510	Absolute change in LDL-C level from baseline to day 510; time-adjusted percentage change in LDL-C level from day 90 to day 540; safety and tolerability of Inclisiran
ORION-4	Patients with ASCVD or high risk (global)	55–80 years old, with ASCVD or high risk (such as diabetes, chronic kidney disease or peripheral artery disease), LDL-C ≥ 1.8 mmol/L (70 mg/dL), optimized lipid-lowering therapy ≥30 days	Pregnant or lactating women, liver dysfunction, other serious diseases or conditions unsuitable for trial participation	Randomized, placebo-controlled, multicenter phase III trial to evaluate the effect of Inclisiran on cardiovascular outcomes. Randomization ratio of 1:1	At least 4 years of follow-up, Observe and test every 6 months	Composite endpoint of coronary heart disease death, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke or coronary revascularization (major adverse cardiovascular events, MACE)	Other cardiovascular outcomes and safety and tolerability of Inclisiran
ORION-18	Patients with primary hypercholesterolemia or mixed hyperlipidemia (Asia)	18–85 years old -with primary hypercholesterolemia or mixed hyperlipidemia -LDL-C ≥ −2.6 mmol/L (100 mg/dL) -maximum tolerated dose of statin ± other lipid-lowering drugs ≥30 days	Pregnant or lactating women -liver dysfunction -other serious diseases or conditions unsuitable for trial participation	Double-blind -randomized -placebo-controlled -multicenter phase III trial to evaluate the efficacy and safety of Inclisiran in Asian populations Randomization ratio of −1:1	Observation and measurement on day, 0, day, 90, day, 180, day, 270, day, 360, day, 450 and day, 540, for a total of, 18 months	Percentage change in LDL-C level from baseline to day,510	Other lipid parameters and safety and tolerability of Inclisiran

HeFH, heterozygous familial hypercholesterolemia; LDL-C, Low-Density Lipoprotein Cholesterol; ASCVD, atherosclerotic cardiovascular disease.

compared to the placebo group, which was statistically significant and clinically meaningful (Leiter et al., 2019; Jahangir et al., 2021). The reduction was consistent and sustained across all subgroups and time points. Inclisiran also reduced other lipid parameters, such as non-HDL-C, apolipoprotein B and lipoprotein (a). Inclisiran was well tolerated and had a similar safety profile to placebo. The most common adverse events were injection site reactions, which were mild or moderate and self-limiting.

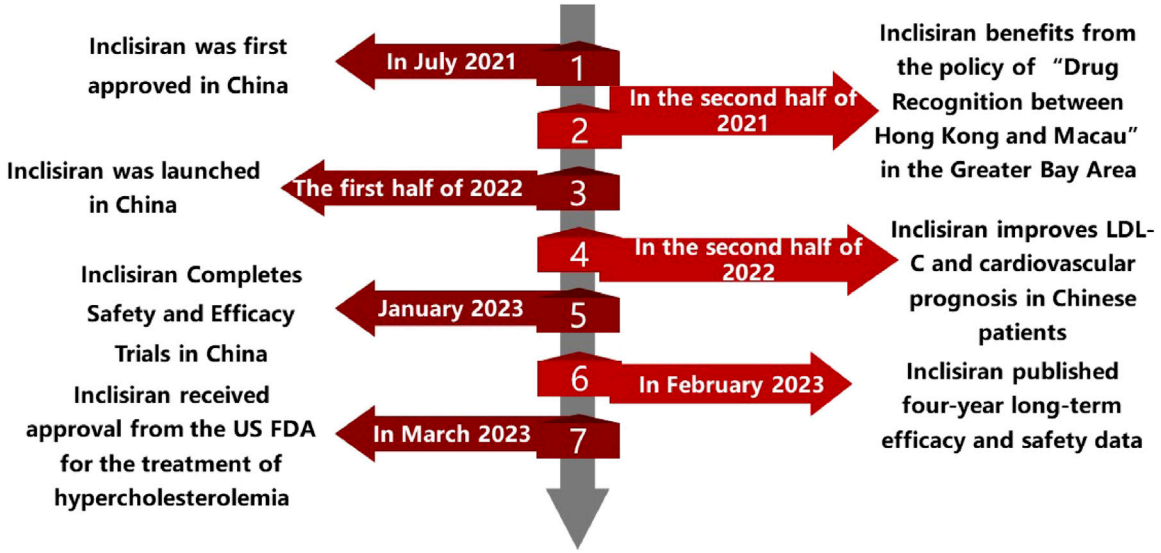

FIGURE 2
The development and use of Inclisiran in China: a chronological overview.

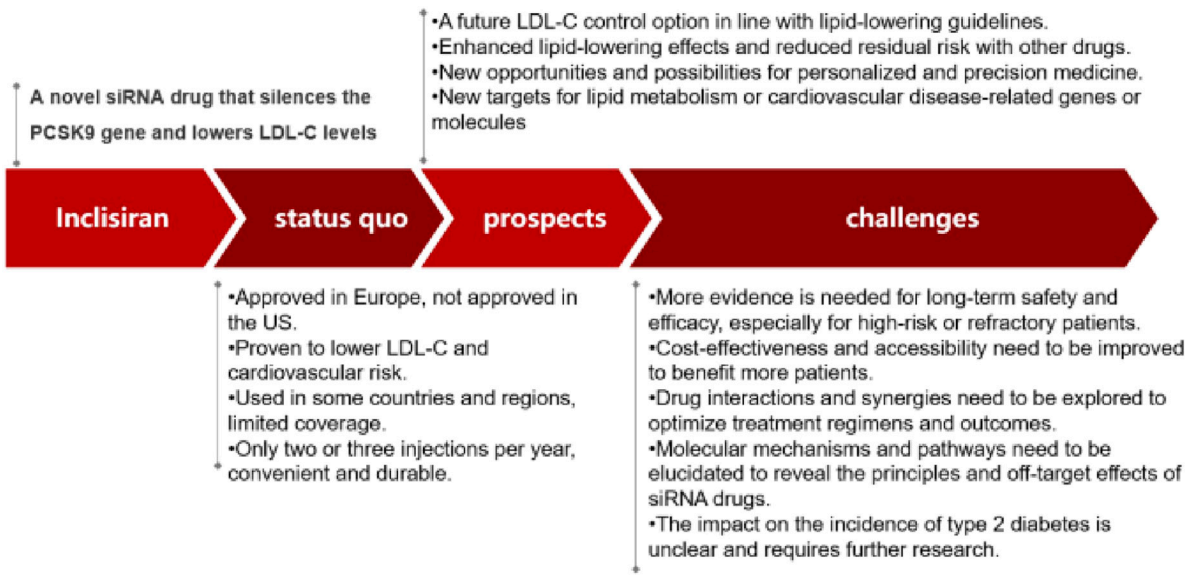

FIGURE 3
Status quo, prospects and challenges of Inclisiran.

4.1.3 ORION-18: inclisiran for asian population

Inclisiran's phase III clinical study-ORION-18, which is also taking place in China and other Asian countries for the first time. This study will enroll about 1,500 patients with primary hypercholesterolemia or mixed dyslipidemia who are not adequately controlled by statins alone or in combination with other lipid-lowering therapies. The primary endpoint is the percentage change in LDL-C levels from baseline to day 510. This study will provide more evidence for the efficacy and safety of Inclisiran in Asian populations, who may have different genetic and environmental factors that affect their lipid metabolism and response to treatment (Jahangir et al., 2021; Ray et al., 2023).

4.1.4 ORION 4: inclisiran for cardiovascular outcomes

Additionally, ORION4 studies are being conducted to further assess the long-term effectiveness, safety, and cardiovascular benefits of Inclisiran (Brandts and Ray, 2021). This is a large-scale, multicenter, randomized trial that will enroll about 15,000 patients with ASCVD or high risk of ASCVD who have

elevated LDL-C levels despite optimal lipid-lowering therapy. It will compare the effects of Inclisiran versus placebo on cardiovascular outcomes. The primary outcome is the composite of coronary heart disease death, non-fatal myocardial infarction, fatal or non-fatal ischemic stroke or coronary revascularization. These are major adverse cardiovascular events (MACE) that can cause significant morbidity and mortality. The trial will follow the patients for at least 4 years and the results could be announced in 2025. This trial will provide important evidence for the long-term benefits and safety of Inclisiran in reducing cardiovascular risk.

A comprehensive summary of the details and findings of the clinical trials of Inclisiran for various hypercholesterolemia populations is given in the table below (See Table 1).

4.2 Safety of inclisiran

4.2.1 Advantages of inclisiran safety profile

Compared with other lipid-lowering therapies, Inclisiran has a low risk of adverse events and no major safety concerns. The most common adverse event reported in the Inclisiran group was injection site reaction, which occurred in about 5% of the patients. However, these reactions were mostly mild and transient, and none of them were severe or persistent. Injection site reactions may be related to the lipid nanoparticles used for siRNA delivery and may decrease with repeated administration. Moreover, Inclisiran did not cause any significant toxicity in liver, kidney, muscle and platelet functions. Laboratory examination showed that there was no change in the levels of alanine aminotransferase, aspartate aminotransferase, creatinine, creatine kinase or platelet count after Inclisiran treatment. Inclisiran also did not affect the cardiovascular system. There was no difference in blood pressure, heart rate or electrocardiogram parameters between the Inclisiran group and the placebo group. Therefore, Inclisiran appears to be a safe and well-tolerated drug for lowering LDL-C levels in patients with hypercholesterolemia or mixed dyslipidemia (Hardy et al., 2021; Wright et al., 2021; Ray et al., 2023).

4.2.2 Adverse reactions of inclisiran and safety comparison with siRNA drugs

One of the concerns about siRNA drugs is their possible off-target effects and immune responses (Meng and Lu, 2017; Hu et al., 2020). However, Inclisiran has been developed to reduce these risks by using a highly specific and stable siRNA sequence and a GalNAc conjugate that enhances the delivery to liver cells (Hardy et al., 2021). Inclisiran has a good overall safety profile (Wright et al., 2020). However, recently, there have been some reports that Inclisiran may have adverse reactions such as diarrhea and headache, which has aroused concern about its long-term safety. Further studies could continue monitoring its long-term safety across diverse populations.

4.3 Comparison with PCSK9 monoclonal antibody therapy

Compared with PCSK9 monoclonal antibodies, Inclisiran acts downstream by degrading mRNA to achieve sustained suppression of PCSK9, whereas monoclonal antibodies directly block the binding of PCSK9 to LDL receptors (Leiter et al., 2019). The dosing frequency of Inclisiran is once or twice yearly, which is significantly less frequent than the 2-week or monthly injections required for monoclonal antibodies (Ray et al., 2020). This may greatly improve patient compliance. In addition, Inclisiran provides more durable lipid-lowering effects, with a single dose maintaining efficacy for 3–6 months. Overall, as an siRNA therapy, Inclisiran has unique advantages in mechanism, longer duration of action, and more convenient administration compared to monoclonal antibodies. Compared with PCSK9 monoclonal antibodies, Inclisiran demonstrated more durable lipid-lowering effects, which was confirmed by the long-term results of ORION-3 (Ray et al., 2023). The treatment course of PCSK9 monoclonal antibodies is generally 1-2 years, while Inclisiran can maintain the effect for up to 4 years. This may make Inclisiran more suitable for long-term use.

4.4 The applications of inclisiran in China nowadays

In addition to conducting clinical trials and obtaining approval in developed countries such as Europe and America, Inclisiran has also initiated a series of investigations and applications in China. However, there are still concerns about the cost and affordability and accessibility of Inclisiran. Inclisiran is priced high, costing about 20,000 yuan per injection, and about 40,000 yuan per year. The drug has not been included in the medical insurance list yet, so patients have to pay for it themselves. The high price of Inclisiran may limit its widespread use in China, which requires improving its accessibility by controlling drug costs and negotiating with medical insurance. We look forward to the real-world application data in the future, to evaluate the cost-effectiveness of Inclisiran in China.

4.4.1 Inclisiran's benefits for Chinese patients

In the near future, this drug will be more widely available in more cities and hospitals, which will have a positive effect on the quality of life of the Chinese people. Inclisiran is expected to benefit millions of Chinese patients with hypercholesterolemia or mixed dyslipidemia who are not adequately controlled by current therapies (Luo et al., 2023).

4.4.2 Inclisiran's entry into China

On July 2 2021, Novartis Pharmaceuticals held a press conference to announce that its new siRNA lipid-lowering drug, Inclisiran, had completed the first injection in China in Boao Super Hospital. The drug was subsequently used in some private hospitals in the Greater Bay Area as the 17th drug approved through the policy of "drug communication between Hong Kong and Macao". This policy allows the use of drugs that have been approved by the regulatory authorities of Hong Kong or Macao in designated medical institutions in Hainan province without additional approval from the mainland authorities. In this way, the policy can facilitate the access of innovative drugs to Chinese patients and promote the development of the healthcare industry in Hainan. The following timeline (Figure 2) summarizes the main events and dates of the introduction and utilization of Inclisiran in China.

5 Discussion

In this review, Inclisiran had been introduced as a novel siRNA lipid-lowering agent that can silence PCSK9 gene expression and lower LDL-C levels with only two or three injections per year. The characteristics and prospects of Inclisiran in terms of its clinical trials, safety and applications in China had also been summarized. In this section, some of the remaining issues and challenges for Inclisiran had been discussed, such as its impact on the risk of type 2 diabetes, its long-term safety and efficacy, its cost-effectiveness and accessibility, and its interactions and synergies with other drugs.

One of the issues that need to be further investigated is the impact of Inclisiran on the risk of type 2 diabetes. Up-regulating LDL receptors in order to reduce LDL-C levels had been shown to have mixed results in terms of its effect on type 2 diabetes incidence. Up-regulating LDL receptors in order to reduce LDL-C levels has been shown to have mixed results in terms of its effect on type 2 diabetes incidence (Ray et al., 2023). Type 2 diabetes is a metabolic disorder that can increase the risk of cardiovascular complications. Some studies suggest that it has a beneficial effect, while others find no significant effect. It is possible that the discrepancy between studies may be due to gender differences, as some studies have reported that women may have a higher risk of developing type 2 diabetes after LDL receptor upregulation than men (Shi et al., 2020). Mendelian randomization had previously proved that the reduction of LDL-C due to genetic variation at PCSK9 does increase the risk of type 2 diabetes (Schmidt et al., 2017). At present, the exact mechanism by which PCSK9 inhibitors increase the risk of type 2 diabetes is unclear. In the same way, it remained to be further discussed whether Inclisiran increased the prevalence of type 2 diabetes.

We believed that the impact of Inclisiran on the risk of type 2 diabetes is an unresolved issue, which may be influenced by gender, genotype and other drugs, and requires more research on individualized, precision and combination therapy to elucidate its mechanism and optimize its application.

Another issue that needs to be addressed is the long-term safety and efficacy of Inclisiran. The results of the clinical trial studies published so far confirmed the efficacy of Inclisiran in lowering LDL-C. However, even with lipid-lowering therapy, there are still some considerable residual risks of cardiovascular disease. These risks are considered to be caused by dysglycemia, hypertension, procoagulant state and inflammation. These are factors that can impair the endothelial function and promote the development of atherosclerosis. Clinical findings regarding lipids have emerged in recent years, and as a result, consensus lipid management guidelines have proposed lower LDL-C control goals. These guidelines are based on the evidence that lower LDL-C levels can reduce the risk of cardiovascular events and mortality in patients with dyslipidemia. Lipid management was gradually changing from "Lower is Better" to "Lower for Longer." This means that achieving and maintaining low LDL-C levels for a long period of time is more important than short-term fluctuations (Ray et al., 2023). We believed that the long-term safety and effectiveness of Inclisiran is an important and uncertain issue, and more follow-up and observation are needed to assess its impact on the prevention and treatment of cardiovascular disease.

A third issue that needs to be considered is the cost-effectiveness and accessibility of Inclisiran. However, there were still some challenges and limitations for Inclisiran and other siRNA drugs. These drugs can effectively lower LDL-C levels by inhibiting the protein that degrades LDL receptors in the liver. The low frequency of treatment of Inclisiran could improve patients' compliance and significantly reduce the complication incidence, which may provide a future solution required for LDL-C-lowering treatment. For instance, the long-term safety and efficacy of Inclisiran need to be further evaluated in larger and more diverse populations, especially in patients with high cardiovascular risk or familial hypercholesterolemia. These are patients who may have more severe or resistant forms of dyslipidemia and may require more intensive or novel treatments. Moreover, the cost-effectiveness and accessibility of Inclisiran need to be improved to make it more affordable and available for patients who need it. The current price and supply of Inclisiran might limit its widespread use and adoption in clinical practice (Desai et al., 2022).

A final issue that needs to be explored is the potential interactions and synergies between Inclisiran and other lipid-lowering agents or cardiovascular drugs. There may be additional or synergistic effects of combining Inclisiran with other drugs that can lower LDL-C levels by different mechanisms or that can modulate other cardiovascular risk factors. The cost-effectiveness and accessibility of Inclisiran is an issue to consider, but Inclisiran and other siRNA drugs still face some challenges and limitations that need to be overcome or addressed.

Additionally, the molecular mechanisms and pathways involved in Inclisiran-mediated LDL-C reduction and cardiovascular protection need to be elucidated to reveal the underlying biology and pharmacology of siRNA drugs. This might help to understand the mode of action and the potential off-target effects of Inclisiran and other siRNA drugs. Finally, the development of novel siRNA drugs targeting other genes or molecules related to lipid metabolism or cardiovascular disease might offer new opportunities and possibilities for personalized and precision medicine. These drugs may provide more specific and tailored treatments for different subtypes or phenotypes of patients with dyslipidemia or cardiovascular disease (Scicchitano et al., 2021; Mercep et al., 2022).

6 Conclusion

In conclusion, Inclisiran was a promising siRNA lipid-lowering agent that can silence PCSK9 gene expression and lower LDL-C levels with only two or three injections per year. It had shown favorable results in various clinical trials for different types of hypercholesterolemia and cardiovascular disease patients. It had also demonstrated a good safety profile and a potential application in China. Globally, Inclisiran has been approved by the US FDA and the European EMA for the treatment of primary hypercholesterolemia or mixed dyslipidemia. In China, Inclisiran has been approved for the general evaluation of drugs in Hainan Province, and some private hospitals can use this innovative lipid-regulating drug. It is expected that Inclisiran will obtain the approval of China NDAs in the near future. However, there were still some issues and challenges that need to be addressed for Inclisiran, such as its impact on the risk of type 2 diabetes, its long-term safety and

efficacy, its cost-effectiveness and accessibility, and its interactions and synergies with other drugs. Moreover, the molecular mechanisms and pathways involved in Inclisiran-mediated LDL-C reduction and cardiovascular protection need to be elucidated, and the development of novel siRNA drugs targeting other genes or molecules related to lipid metabolism or cardiovascular disease may offer new opportunities and possibilities for personalized and precision medicine. Compared with PCSK9 antibodies, Inclisiran had the advantages of less frequent administration, lower injection volume, and potentially lower cost. However, it should be noted that Inclisiran had not yet proven its ability to reduce cardiovascular events and mortality in large-scale trials, while PCSK9 antibodies had already demonstrated such benefits in several studies. Therefore, the clinical outcomes of Inclisiran need to be further confirmed by ongoing or future trials. To summarize the main points of our review, we had provided a graphical abstract in Figure 3 (See Figure 3).

7 Prospects

Recently, the indication of Inclisiran has been expanded to populations with high cardiovascular risk factors but without a history of cardiovascular events, for primary prevention. This expanded indication provides a new option for early intervention in high-risk populations. The sustained lipid-lowering effect and convenient dosing regimen of Inclisiran may help improve medication adherence and therapeutic outcomes in these patients. This opens up a promising primary prevention market for Inclisiran. We look forward to more clinical study results to verify the long-term safety and efficacy of Inclisiran in diverse populations.

Author contributions

YZ: Conceptualization, Methodology, Writing–original draft. HC: Conceptualization, Software, Visualization, Writing–review and editing. LH: Funding acquisition, Supervision, Writing–review and editing. HW: Project administration, Supervision, Writing–review and editing. BL: Project administration, Supervision, Writing–review and editing. MZ: Project administration, Writing–review and editing. JL: Software, Writing–review and editing. LY: Conceptualization, Methodology, Writing–review and editing, Writing–original draft. FL: Conceptualization, Methodology, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Ismail Laher,
University of British Columbia, Canada

REVIEWED BY
Yuan Xinxu,
Virginia Commonwealth University, United States
Piyushkumar R. Kapopara,
Thermo Fisher Scientific, United States

*CORRESPONDENCE
Wei Qin,
qinwei@mail.jnmc.edu.cn

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Anti-atherosclerosis mechanisms associated with regulation of non-coding RNAs by active monomers of traditional Chinese medicine

Guoqing Liu[1,2], Liqiang Tan[3], Xiaona Zhao[2,4], Minghui Wang[1,2], Zejin Zhang[2,5], Jing Zhang[2], Honggang Gao[2], Meifang Liu[2] and Wei Qin[2]*

[1]School of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan, Shandong, China, [2]School of Pharmacy, Jining Medical University, Rizhao, Shandong, China, [3]Department of Nasopharyngeal Carcinoma, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong, China, [4]School of Pharmacy, Weifang Medical University, Weifang, Shandong, China, [5]School of Pharmacy, Binzhou Medical University, Yantai, Shandong, China

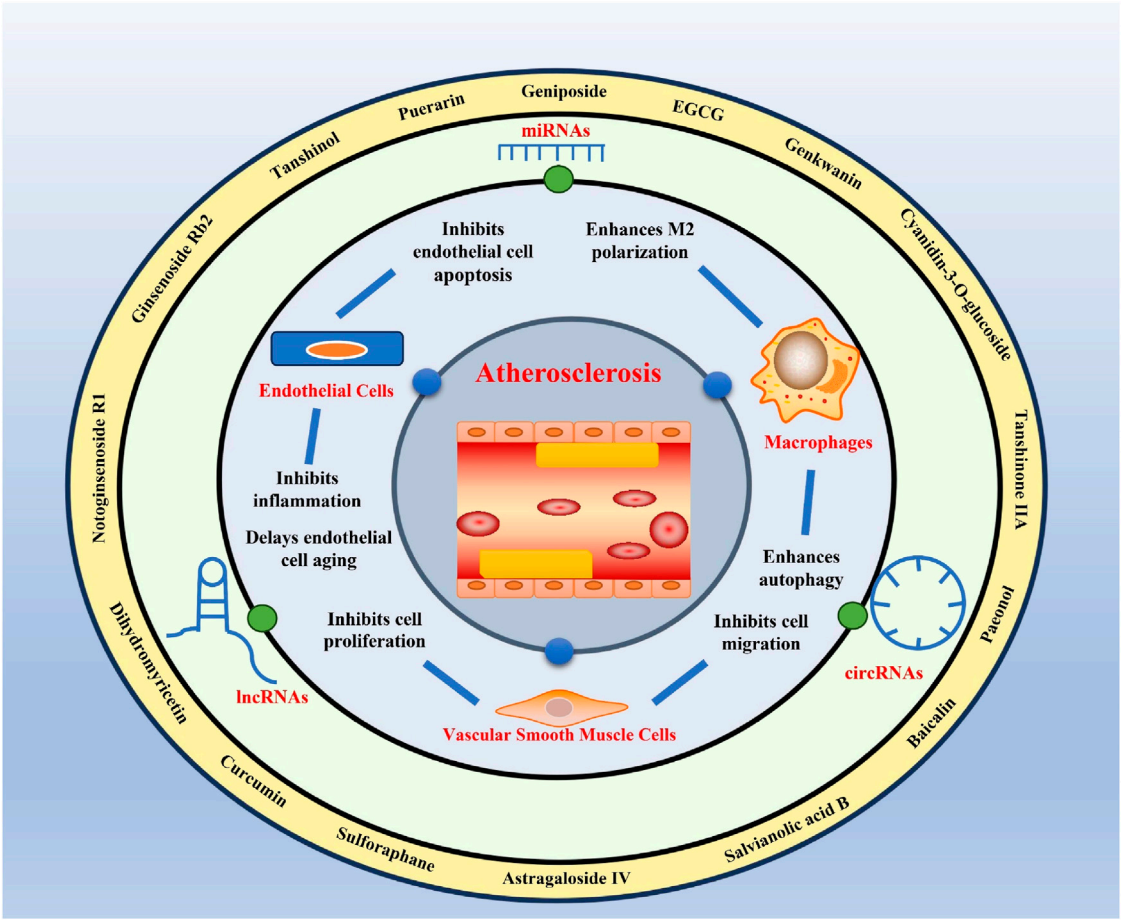
Atherosclerosis is the leading cause of numerous cardiovascular diseases with a high mortality rate. Non-coding RNAs (ncRNAs), RNA molecules that do not encode proteins in human genome transcripts, are known to play crucial roles in various physiological and pathological processes. Recently, researches on the regulation of atherosclerosis by ncRNAs, mainly including microRNAs, long non-coding RNAs, and circular RNAs, have gradually become a hot topic. Traditional Chinese medicine has been proved to be effective in treating cardiovascular diseases in China for a long time, and its active monomers have been found to target a variety of atherosclerosis-related ncRNAs. These active monomers of traditional Chinese medicine hold great potential as drugs for the treatment of atherosclerosis. Here, we summarized current advancement of the molecular pathways by which ncRNAs regulate atherosclerosis and mainly highlighted the mechanisms of traditional Chinese medicine monomers in regulating atherosclerosis through targeting ncRNAs.

KEYWORDS

non-coding RNAs, traditional Chinese medicine, atherosclerosis, cardiovascular diseases, active monomer

1 Introduction

Atherosclerosis is characterized by fibro-fat lesions on the walls of arteries with extremely high morbidity and mortality rate (Libby et al., 2019). Atherosclerosis is considered to be an important pathological basis for cerebrovascular and cardiovascular diseases such as cerebral infarction, coronary heart disease and myocardial infarction (Libby, 2021). There are many causes of atherosclerosis, such as inflammatory reactions (Zhu et al., 2018), cell death and aging (Bazioti et al., 2022), and endothelial to mesenchymal transition (Liang et al., 2022), among which chronic inflammation is the reason that has been frequently studied in the past few years. At the molecular level, telomere damage, genomic DNA damage, and mitochondrial DNA damage accumulate in vascular endothelial cells, which induce endothelial cell aging and chronic inflammation.



GRAPHICAL ABSTRACT

Persistent inflammation results in increased accumulation of lymphocytes and macrophages, leading to atherosclerosis (Wang and Bennett, 2012; Okuyama et al., 2015; Ruparelia and Choudhury, 2020). The pathogenesis and therapeutic targets of atherosclerosis have long been the focus in the field of cardiovascular researches. Statins, inhibitors of the hydroxymethylglutaryl-CoA (HMG-CoA) reductase enzyme, are powerful cholesterol lowering medications and the most commonly used clinical drugs for the prevention and treatment of atherosclerosis (Okuyama et al., 2015). Statins can reduce morbidity and mortality in patients with cardiovascular diseases. However, statins may affect the drug-drug interactions because different safety and tolerability, especially when in combination with other cardiovascular drugs, which will lead to increased risk of statin-associated hepatotoxicity and myopathy (Bellosta and Corsini, 2018). Therefore, it is urgent to discover new therapeutic targets and new drugs for atherosclerosis.

RNAs in mammalian cells are complex, some of which have the function of encoding proteins, but some of which lack this function. At present, the RNAs that lack the function of encoding proteins are named as non-coding RNAs (ncRNAs), of which the most studied are microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) (Lu and Rothenberg, 2018; Chen et al., 2021a; Bridges et al., 2021). NcRNAs have been proved to play important regulatory roles in pathogenesis of atherosclerosis through affecting inflammatory reaction, cell activation and proliferation, and lipid metabolism (Feinberg and Moore, 2016; Aryal and Suarez, 2019). Nowadays, therapeutic strategies targeting ncRNAs have entered the clinical testing phase for the treatment of cancers and have been considered as an attractive approach for the treatment of atherosclerosis.

Since ancient times, numerous herbal medicines have been used for the treatment of atherosclerosis-related diseases and decoction is the main form of traditional Chinese medicine used in clinic. With the development of separation technology, it has become possible to separate more pharmacologically active monomers from traditional Chinese medicine. This allows researchers to conduct pharmacological studies on the specific monomers rather than the whole of medicinal plants. At present, studies have found that many active monomers of traditional Chinese medicine have positive effects on atherosclerosis, such as saponins (Luo et al., 2022), flavonoids (Park et al., 2006) and alkaloids (Li et al., 2021d). For example, berberine, an active ingredient extracted from *Berberis aristata* DC., can activate PPAR-γ pathway in macrophages, resulting in decreased expressions of inflammatory factors like monocyte chemoattractant protein-1 (MCP-1) and tumor

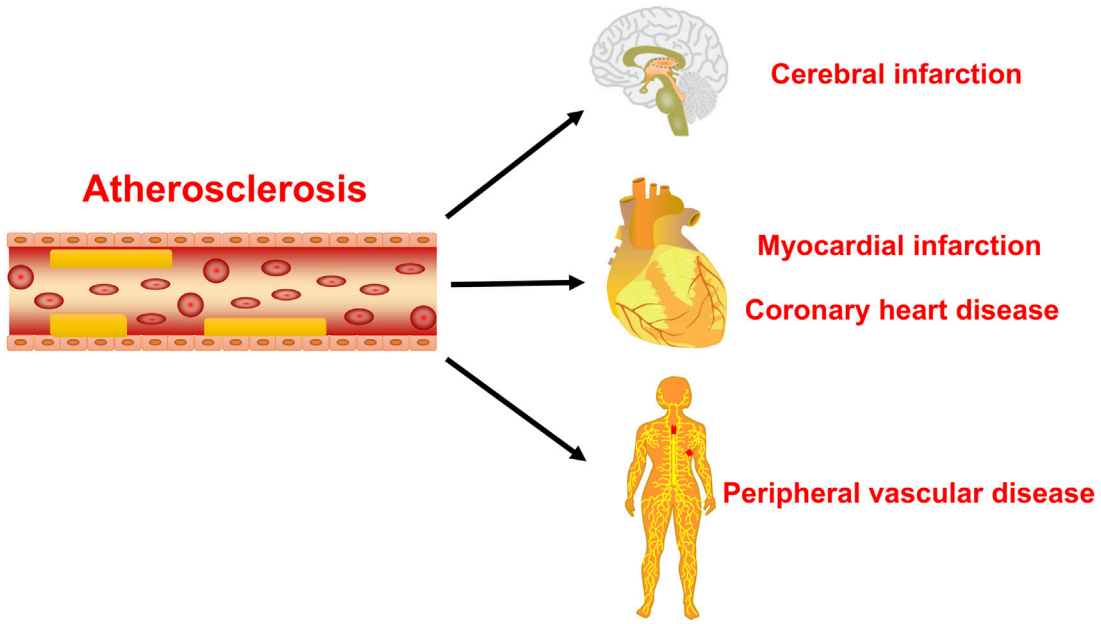


FIGURE 1
The main hazards of atherosclerosis. The atherosclerosis is the main cause of coronary heart disease, cerebral infarction, and peripheral vascular disease.

necrosis factor-α (TNF-α) (Chen et al., 2008). Another study found that hydroxysafflor yellow A, a natural compound from *Carthamus tinctorius L.*, exerts protective effects on atherosclerosis by suppressing vascular endothelial cell dysfunction, vascular smooth muscle cell proliferation and migration, foam cell formation, and platelet activation (Xue et al., 2021). These active monomers of traditional Chinese medicine are predicted to have high therapeutic potential in atherosclerosis treatment. However, the specific drug targets of these active ingredient are not fully understood, which limits clinical application.

Recently, more and more studies have found that ncRNAs are the key mediators of the pharmacological effect of traditional Chinese medicine. Here, we summarized current advances of mechanisms of ncRNAs in regulating atherosclerosis. Furthermore, we highlighted the current advances in the active monomers of traditional Chinese medicine which have atheroprotective effects by regulating ncRNAs.

2 The role of ncRNAs in regulating atherosclerosis

2.1 miRNAs and atherosclerosis

MiRNAs (typically 20–25 nucleotides) are single-stranded RNA molecules that can bind to complementary sequences within the 3′ untranslated region of mRNA targets. Once the miRNA binds to the mRNA, it can degrade the mRNA via cleavage or inhibit the translation of mRNAs into proteins (Winter et al., 2009; Thum and Mayr, 2012). MiRNAs are the most studied ncRNAs in atherosclerosis and have been shown to regulate the fate and function of atherosclerosis associated cells, including endothelial cells, inflammatory cells, and vascular smooth muscle cells (VSMCs). MiRNAs can affect endothelial cell function by exacerbating senescence of endothelial cells, which is considered as a key mechanism of atherosclerosis (Menghini et al., 2009; Fiedler and Thum, 2016). There are many miRNAs involved in the regulation of endothelial cell senescence, such as miR-146a and miR-217 (Wang et al., 2021; Xiao et al., 2021). Studies have found that mesenchymal stem cell-derived extracellular vesicles attenuate endothelial cell senescence by regulating miR-146a/Src signaling (Xiao et al., 2021). MiR-217 can also promote endothelial cell senescence through the SIRT1/p53 signaling pathway (Wang et al., 2021). In addition, miRNAs can control the inflammatory state of the vasculature by affecting leukocyte activation and infiltration (Perez-Sanchez et al., 2017; Pankratz et al., 2018). In the setting of atherosclerosis, miR-126 promotes macrophage polarization to the M2 phenotype by downregulating VEGFA and krüppel-like factor 4 (KLF4) (Shou et al., 2023). MiRNAs have also been shown to affect foam cell formation and subsequent plaque formation (Eken et al., 2017; Maitrias et al., 2017). MiR-302a has been shown to promote the formation of foam cells and increase the outflow of cholesterol in macrophage by increasing ATP-binding cassette transporter A1 (ABCA1) activity (Meiler et al., 2015). In addition, the function of VSMCs can also be regulated by miRNAs. For example, miR-146b-5p reduces the expression of its target genes Bag1 and Mmp16, thereby affecting the proliferation and migration of VSMCs during atherosclerosis (Sun et al., 2020). A study also found that miR-374 may be a potential biomarker for the diagnosis of atherosclerosis, and overexpression of miR-374 promotes the proliferation and migration of VSMCs (Wang et al., 2020b). MiR-663 can target

TABLE 1 Active monomers of traditional Chinese medicine and their ncRNA targets.

Active monomers	ncRNA	Target genes	Related hallmark	Model	References
Geniposide	miR-101	MKP-1	Inhibits inflammation	*In vivo*: ApoE$^{-/-}$ mice	Cheng et al. (2019)
				In vitro: RAW264.7	
	miR-21	PTEN	Inhibits inflammation and oxidative stress	*In vitro*: HUVECs	Zhou et al. (2020)
Astragaloside IV	circ-0000231	miR-135a-5p	Inhibits apoptosis, inflammation, and oxidative stress; Promotes the viability and migration ability	*In vitro*: HUVECs	Shao et al. (2021)
	miR-33a	ABCA1	Promotes the outflow of cholesterol	*In vivo*: ApoE$^{-/-}$ mice	Qin et al. (2018)
				In vitro: THP-1	
	miR-17-5p	PCSK9/VLDLR	Inhibits inflammation	*In vivo*: ApoE$^{-/-}$ mice	Qin et al. (2022)
				In vitro: VSMCs	
	lncRNA H19	DUSP5	Inhibits autophagy and mineralization	*In vivo*: ApoE$^{-/-}$ mice C57BL/6J mice	Song et al. (2019)
				In vitro: HASMCs	
Notoginsenoside R1	miR-147a	MyD88	Inhibits inflammation and oxidative stress	*In vitro*: HUVECs	Li and Huang (2021)
	miR-221-3p	TLR4	Inhibits apoptosis, inflammation, and oxidative stress	*In vitro*: HUVECs	Zhu et al. (2020)
	miR-34a	SIRT1	Delays aging	*In vitro*: HUVECs	Lai et al. (2018)
Tanshinone IIA	miR-130b	WNT5A	Inhibits inflammation and adipogenesis	*In vitro*: THP-1	Yuan et al. (2020)
	miR-712-5p	?	Inhibits inflammation and cell proliferation	*In vitro*: VSMCs	Qin et al. (2020)
	miR-375	KLF4	Enhances autophagy and M2 polarization of macrophages	*In vivo*: ApoE$^{-/-}$ mice	Chen et al. (2019a)
				In vitro: RAW264.7	
	miR-21-5p	TPM1	Inhibits proliferation and migration	*In vitro*: HASMCs	Jia et al. (2019)
Salvianolic acid B	miR-146a	?	Inhibits proliferation	*In vivo*: Carotid bifurcation ligated mice	Zhao et al. (2019)
				In vitro: VSMCs	
Tanshinol	lncRNA TUG1	miR-26a	Inhibits apoptosis	*In vivo*: ApoE$^{-/-}$ mice	Chen et al. (2016)
				In vitro: HAECs, ECV304 cells	
Genkwanin	miR-101	MKP-1	Inhibits inflammation	*In vitro*: RAW264.7	Gao et al. (2014)
Dihydromyricetin	miR-21	DDAH1	Increases NO production and weakens endothelial dysfunction	*In vivo*: ApoE$^{-/-}$ mice	Yang et al. (2018), Yang et al. (2020)
				In vitro: HUVECs, THP-1	
Sulforaphane	miR-34a	SIRT1	Reduces oxidative stress	*In vitro*: HUVECs	Li et al. (2021c)
Cyanidin-3-O-glucoside	miR-204-5p	SIRT1	Inhibits inflammation and apoptosis	*In vivo*: Rabbit model of HFD + balloon catheter injury	Wang et al. (2020c)
				In vitro: HUVECs	
Baicalin	miR-126-5p	HMGB1	Inhibits proliferation and migration	*ex vivo*:Blood of atherosclerosis patients and healthy people	Chen et al. (2019b)
				In vitro: VSMCs	
Curcumin	lncRNA MIAT	EZH2	Inhibits inflammation	*In vivo*: ApoE$^{-/-}$ mice	Ouyang et al. (2022)
				In vitro: HUVECs	
	miR-125a-5p	SIRT6	Promotes cholesterol efflux	*In vitro*: THP-1	Tan et al. (2021)

(Continued on following page)

TABLE 1 (*Continued*) Active monomers of traditional Chinese medicine and their ncRNA targets.

Active monomers	ncRNA	Target genes	Related hallmark	Model	References
EGCG	miR-33a	ABCA1	Promotes cholesterol efflux	*In vitro*: THP-1	Yang et al. (2016)
Ginsenoside Rb2	miR-216a	Smad3	Inhibits inflammation and aging	*In vitro*: HUVECs, HAECs	Chen et al. (2021b)
Paeonol	miR-223	STAT3	Inhibits inflammation	*In vivo*: ApoE$^{-/-}$ mice	Liu et al. (2018)
				In vitro: HUVECs, THP-1	
	miR-223	?	Inhibits inflammation	*In vivo*: SD rats	Shi et al. (2020)
				In vitro: RAECs	
	miR-126	VCAM-1	Inhibits monocyte adhesion to endothelial cells	*In vivo*: SD rats	Yuan et al. (2016)
				In vitro: VECs isolated from the thoracic aorta of rats	
	miR-21	PTEN	Inhibits inflammation	*In vivo*: SD rats	Liu et al. (2014)
				In vitro: VECs isolated from the thoracic aorta of rats	
	miR-30a	Beclin-1	Inhibits autophagy	*In vivo*: SD rats	Li et al. (2018a)
				In vitro: VECs isolated from the thoracic aorta of rats	
	miR-338-3p	TET2	Inhibiting apoptosis, inflammation, and oxidative stress	*In vitro*: VECs isolated from the thoracic aorta of mice	Yu et al. (2020b)
Puerarin	miR-29b-3p	IGF1	Inhibits inflammation and proliferation	*In vivo*: ApoE$^{-/-}$ mice	Li et al. (2023)
				In vitro: hVSMCs	

MKP-1, mitogen-activated protein kinase phosphatase 1; PTEN, phosphatase and tensin homolog, ABCA1 ATP-binding cassette transporter A1, PCSK9 proprotein convertase subtilisin/kexin type 9, VLDLR, very low-density lipoprotein receptor, KLF4 krüppel-like factor 4, DUSP5 dual specificity phosphatase 5, MyD88 myeloid differentiation primary response 88, TLR4 toll-like receptor 4, SIRT1 sirtuin-1, p53 tumor protein 53, WNT5A wingless/integrated-5A, TPM1 tropomyosin 1, DDAH1 dimethylarginine dimethylaminohydrolase 1, HMGB1 high mobility group box 1 protein, EZH2 enhancer of zeste homolog 2, Smad3 sma- and mad-related protein 3, STAT3 signal transducer and activator of transcription 3,VCAM-1, Vascular cell adhesion molecule-1, IGF1 insulin-like growth factor 1, ApoE$^{-/-}$mice apolipoprotein e-knockout mice, RAW264.7 RAW, 264.7 mouse leukemia macrophage cell line, HUVECs, human umbilical vein endothelial cells; THP-1, human acute monocytic leukemia cell line; VSMCs, vascular smooth muscle cells; HASMCs, human aortic vascular smooth muscle cells; HAEC, human aortic endothelial cells; SD, rats sprague-dawley rats; RAECs, rat aortic endothelial cells; VECs, vascular endothelial cells; hVSMCs, human vascular smooth muscle cells, TET2 tet methylcytosine dioxygenase 2.

high mobility group AT-hook 2 (HMGA2) to inhibit the proliferation of VSMCs, thereby delaying the development of atherosclerosis (Deng and Li, 2022). In conclusion, miRNAs regulate atherosclerosis through affecting the function of endothelial cells, macrophages, and VSMCs.

2.2 lncRNAs and atherosclerosis

LncRNAs are ncRNAs longer than 200 nucleotides (Di Mauro et al., 2018), which are abnormally expressed in many pathological tissues (Li et al., 2020; Zang et al., 2020). Unlike miRNAs, the actions of lncRNAs are relatively complex. LncRNA can be a source of miRNA. For example, miR-31 gene is embedded within an intron of the lncRNA LOC554202 and its transcription is regulated by the methylation state of the host gene promoter (Augoff et al., 2012). Morover, lncRNAs can bind to DNA, mRNA and proteins to regulate their expressions or functions (Guttman and Rinn, 2012). The most widely known mechanism is the competitive endogenous RNA (ceRNA), in which way lncRNA acts as a negative regulator of miRNA (Salmena et al., 2011). In recent years, studies have shown that lncRNAs are dynamically expressed in developing and diseased blood vessels, suggesting that lncRNAs have profound biological functions in atherosclerosis (Guo et al., 2019; Simion et al., 2020). LncRNAs can regulate atherosclerosis by influencing the function of vascular cells. For example, lncRNA HOXA11-AS is significantly upregulated in aortic tissue of atherosclerotic mice and oxidized low-density lipoprotein (ox-LDL)-induced endothelial cells. HOXA11-AS knockdown attenuates endothelial injuries by directly regulating the miR-515-5p/ROCK1/eNOS axis (Gao et al., 2022). In addition to endothelial cells, lncRNA also plays a role in atherosclerosis by affecting VSMCs and macrophages. For example, lncRNA TUG1 can promote the proliferation of VSMCs by regulating the miRNA-21/PTEN axis (Li et al., 2018b). LncRNA MAARS interacts with HuR to increase macrophage apoptosis in the blood vessels (Simion et al., 2020). What's more, lncRNA kcnq1ot1 can compete with miR-452-3p to promote macrophage lipid accumulation and accelerate the development of atherosclerosis (Yu et al., 2020a).

2.3 circRNAs and atherosclerosis

CircRNAs are closed circular molecules, which distinguishes them from other linear RNA molecules. CircRNAs were originally considered as by-products of mRNA cleavage, but now they are

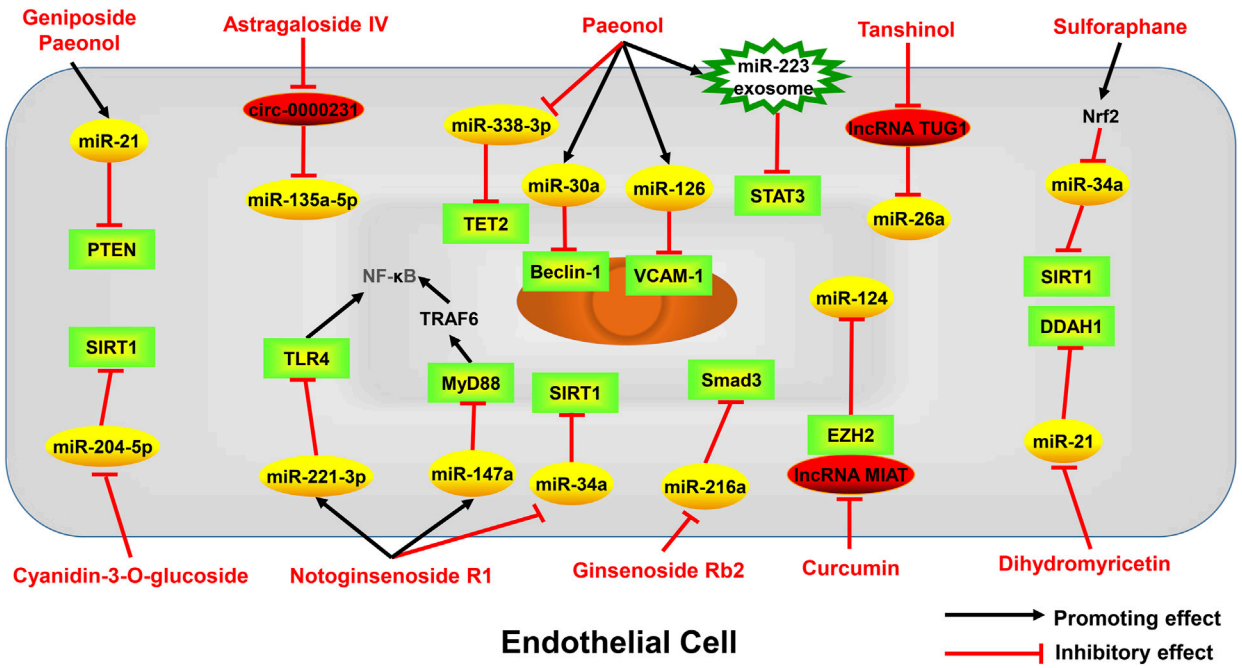


FIGURE 2
Active monomers of traditional Chinese medicine improve the occurrence and development of atherosclerosis through regulating ncRNAs in endothelial cells. PTEN phosphatase and tensin homolog deleted on chromosome ten, VCAM-1 Vascular cell adhesion molecule-1, TLR4 toll-like receptor 4, MyD88 myeloid differentiation primary response 88, TRAF6 TNF receptor-associated factor 6, NF-κB nuclear factor kappa-B, SIRT1 sirtuin-1, DDAH1 dimethylarginine dimethylaminohydrolase 1, EZH2 enhancer of zeste homolog 2, p53 tumor protein p53, Smad3 sma- and mad-related protein 3, STAT3 signal transducer and activator of transcription 3, TET2 tet methylcytosine dioxygenase 2.

thought to be stable and functional ncRNAs (Chen, 2016). Compared to miRNAs, circRNAs are less studied ncRNAs in atherosclerosis. Still, there are studies that have shown circRNAs can regulate the fate and function of atherosclerosis-associated cells, including endothelial cells, macrophages, and VSMCs. As with lncRNAs, circRNAs can also compete with miRNAs as ceRNAs, which is the mostly investigated mechanism (Ren et al., 2021). In endothelial cells, a study demonstrated that circ-RELL1 plays a pro-inflammatory role in endothelial cells by directly binding to miR-6873-3p and subsequently activating NF-κB signaling pathway (Huang et al., 2020). Circ_0086296 induces aberrant endothelial cell phenotypes by spongesizing miR-576-3p, resulting in severe atherosclerotic lesions (Zhang et al., 2022). In VSMCs, circRNA-0044073 promotes the proliferation and invasion of VSMCs by targeting miR-107 and activating the JAK/STAT signaling pathway (Shen et al., 2019). In macrophages, overexpression of circ_0004104 results in dysregulation of atherosclerosis-related genes in THP-1-derived macrophages (Wang et al., 2019). It is noticed that the role of circRNAs in atherosclerosis has rarely been studied, which may become a research hotspot in the future.

Since the role of ncRNAs in atherosclerosis is emerging, they have been considered as potential drug targets in developing therapeutic agents. As we know, traditional Chinese medicine has a long history of treating atherosclerosis in China. In particular, studies have shown that the monomers extracted from traditional Chinese medicine are the main functional components that possess anti-atherosclerotic activity, and these activities can be mediated by ncRNAs.

3 Active monomers of traditional Chinese medicine relieve atherosclerosis by regulating ncRNAs

Nowadays, the researches about the regulation of atherosclerosis by active monomers of traditional Chinese medicine are tremendous. However, the drug targets of traditional Chinese medicine remain unclear, which affects the clinical application of these medicine. It is clear that ncRNAs appear to be important players during atherosclerosis and important targets of traditional Chinese medicine. Therefore, it is particularly important to discover the mechanism by which the active monomers of traditional Chinese medicine relieve atherosclerosis through ncRNAs.

3.1 Geniposide

Geniposide, an iridoid glucoside, is the main active ingredient of *Gardenia jasminoides* J. Ellis. Geniposide exhibits a variety of anti-inflammatory and anti-oxidative functions and has good therapeutic effects on cardiovascular diseases (Fu et al., 2012b). A study has found that geniposide treatment reduces lipid levels and plaque size in the mouse model of atherosclerosis. Mechanistically, geniposide downregulates miR-101 to upregulate mitogen-activated protein kinase phosphatase-1 (MKP-1) and suppresses the production of inflammatory factors in macrophages (Cheng et al., 2019). MiR-21 has been shown to play an important role in regulating inflammatory responses by targeting phosphatase and tensin

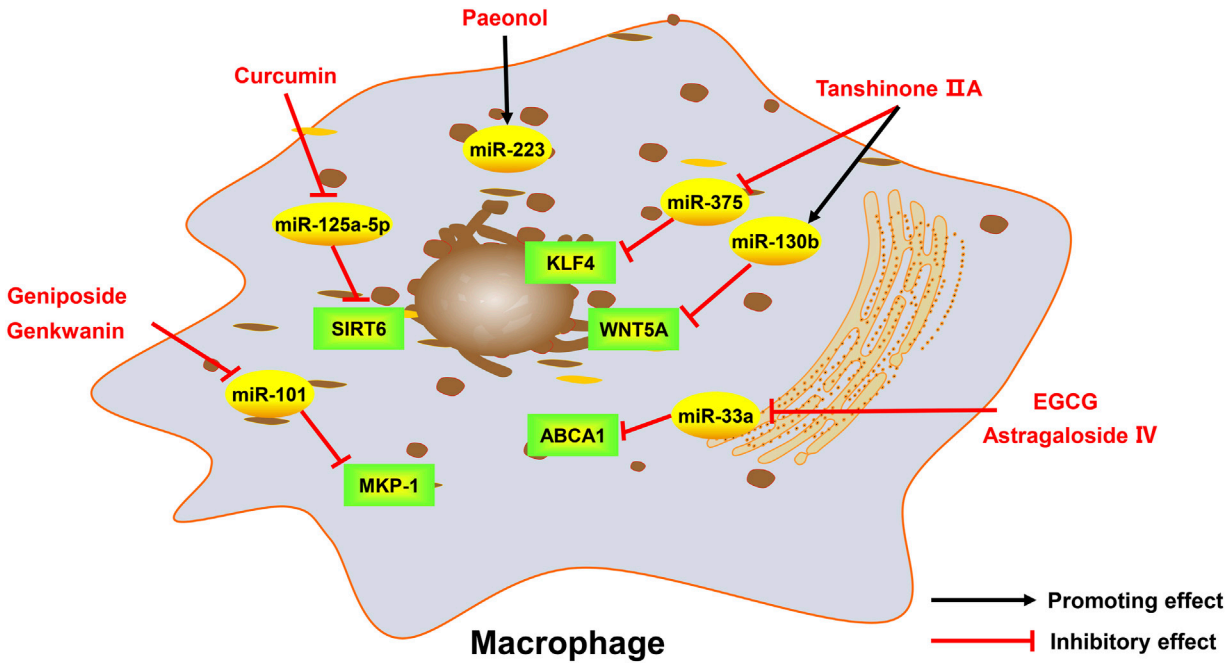


FIGURE 3
Active monomers of traditional Chinese medicine improve the occurrence and development of atherosclerosis through regulating ncRNAs in macrophages. MKP-1 mitogen-activated protein kinase phosphatase 1, ABCA1 ATP-binding cassette transporter A1, WNT5A wingless/integrated-5A, KLF4 krüppel-like factor 4, SIRT6 sirtuin 6.

homolog (PTEN) (Sheedy, 2015; Li et al., 2022). A study established a endothelial cell injury model by using ox-LDL and found geniposide protects endothelial cells from ox-LDL-induced injury by inhibiting oxidative stress and inflammation, and these effects are partly due to the enhancement of the miR-21/PTEN pathway (Zhou et al., 2020). Taken together, miR-101 and miR-21 are involved in the anti-inflammatory effect of geniposide in the setting of atherosclerosis.

3.2 Astragaloside IV

Astragaloside IV is a saponin isolated from *Astragalus membranaceus* (Fisch.) Bunge, which has excellent cardioprotective effects (Tan et al., 2020). Astragaloside IV has been reported to protect endothelial cells from oxidative damage caused by ox-LDL through regulating the LOX-1/NLRP3 signaling pathway (Qian et al., 2019a). Recently, a study found that circ_0000231 is the key downstream target of astragaloside IV, which regulates miR-135a-5p to target chloride intracellular channel 4 (CLIC4), and contributes to the protective role of astragaloside IV in ox-LDL-induced endothelial cell injury (Shao et al., 2021). CLIC4 is also a protein associated with endothelial cell apoptosis (Zhang et al., 2020b), indicating astragaloside IV may also inhibit endothelial cell apoptosis by regulating CLIC4 through circ_0000231. Several miRNAs have been shown to be the targets of astragaloside IV. For example, astragaloside IV can protect cardiomyocytes from hypoxia-induced injury by downregulating miR-23a and miR-92a (Gong et al., 2018). ABCA1, a membrane transporter that mediates cholesterol efflux (Chen et al., 2022), has been proved to be a target of miR-33a (Gao et al., 2018). A study has found that astragaloside IV can promote cholesterol efflux in macrophages and inhibit atherosclerosis through regulating miR-33a/ABCA1 pathway (Qin et al., 2018). The serum miR-17-5p is elevated in patients with atherosclerosis and miR-17-5p knockdown can alleviate atherosclerotic lesions and inhibit the proliferation and migration of VSMCs by directly up-regulating very low density lipoprotein receptor (VLDLR), or indirectly regulate VLDLR by affecting proprotein convertase subtilisin kexin 9 (PCSK9) (Tan et al., 2017). Astragaloside IV has been shown to downregulate miR-17-5p and further affect VLDLR expression, thus inhibiting vascular inflammation (Qin et al., 2022). In addition, lncRNA H19 has also been reported to mediate astragaloside IV's anti-atherosclerotic effect. H19 negatively regulates dual-specificity phosphatase 5 (DUSP5) expression and represses DUSP5/ERK1/2 axis (Tao et al., 2016). Astragaloside IV could attenuate autophagy and mineralization of VSMCs in atherosclerosis by up-regulating H19 and inhibiting DUSP5 (Song et al., 2019). In summary, astragaloside IV can regulate the function of endothelial cells, VSMCs, and macrophages in atherosclerosis by targeting multiple miRNAs, lncRNAs and circRNAs. Therefore, it can be expected that astragaloside IV can exert an excellent anti-atherosclerotic effect through ncRNAs in the clinic.

3.3 Notoginsenoside R1

Notoginsenoside R1, the monomer extracted from *Panax notoginseng* (Burkill) F.H.Chen, has a unique effect of promoting

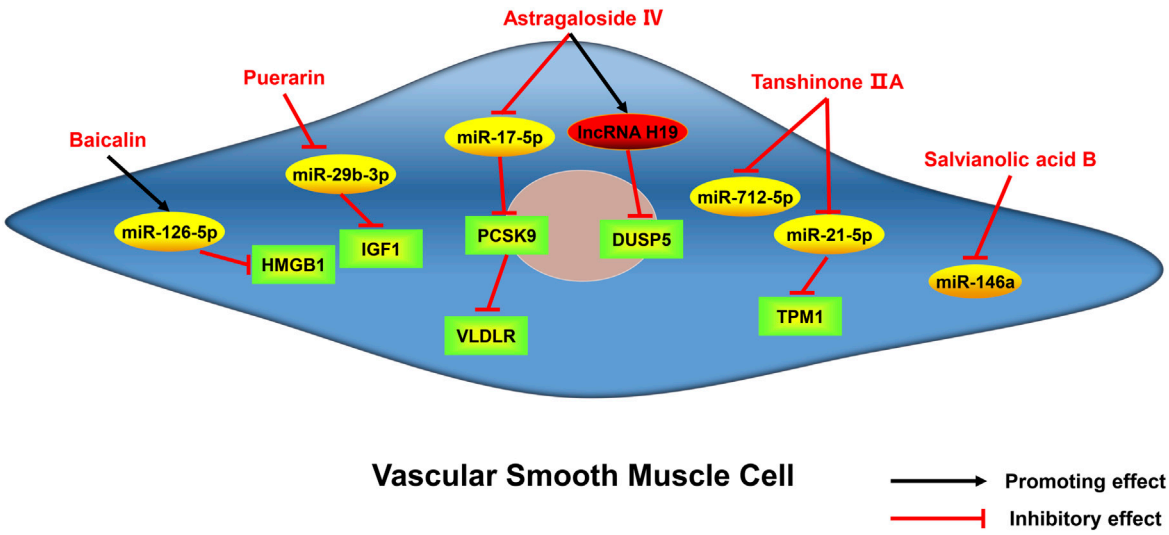


FIGURE 4
Active monomers of traditional Chinese medicine improve the occurrence and development of atherosclerosis through regulating ncRNAs in vascular smooth muscle cells. DUSP5 dual specificity phosphatase 5, PCSK9 proprotein convertase subtilisin/kexin type 9, TPM1 tropomyosin 1, IGF1 insulin-like growth factor 1, HMGB1 high mobility group box 1 protein, VLDLR very low-density lipoprotein receptor.

blood circulation and has been used on clinical treatment of cardiovascular diseases (Lei et al., 2022). Myeloid differentiation primary response gene 88 (MyD88) is an important immunoregulatory factor, and studies have found that inhibiting MyD88 has a good effect on diabetes (Androulidaki et al., 2018). Notoginsenoside R1 was found to relieve high glucose-induced endothelial cell inflammation and oxidative stress by downregulating the MyD88 via up-regulating miR-147a (Li and Huang, 2021). The Toll-like receptor 4 (TLR4)/Nuclear factor-κB (NF-κB) pathway participates in oxidative stress and induces atherosclerosis in ApoE$^{-/-}$ mice by up-regulating inflammatory cytokines (Tang et al., 2015). A study revealed that notoginsenoside R1 could upregulate the expression of miR-221-3p to target TLR4/NF-κB pathway, thereby inhibiting ox-LDL-induced endothelial cell apoptosis, oxidative stress, and inflammation (Zhu et al., 2020). Notoginsenoside R1 may also play a role in delaying senescence of endothelial cells. Notoginsenoside R1 can decrease the expressions of miR-34a and p53, while increase the expression of SIRT1, thus enhancing the intracellular superoxide dismutase (SOD) activity and cell proliferation capacity in hydrogen peroxide-induced endothelial cell aging model (Lai et al., 2018). These studies suggest that notoginsenoside R1 has a strong and multifaceted endothelial protective effect through regulating ncRNAs.

3.4 Tanshinone IIA, salvianolic acid B, tanshinol

Tanshinone, extracted from the traditional Chinese medicine *Salvia miltiorrhiza* Bunge, is a fat-soluble phenanthrene quinone compound with bacteriostatic effect (Wang et al., 2017). Among tanshinone, tanshinone IIA has been clinically proved to have a more significant effect on cardiovascular diseases, especially its anti-inflammatory effect on macrophages. Tanshinone IIA reduces the production of inflammatory factors and adipogenesis in macrophages by up-regulating miR-130b and downregulating WNT5A, thereby relieving the development of atherosclerosis (Yuan et al., 2020). Previous studies have demonstrated that miR-712 is involved in atherosclerosis-related pathological processes, such as VSMCs calcification and endothelial cell inflammation (Son et al., 2013). Tanshinone IIA can inhibit VSMCs inflammation and proliferation by inhibiting miR-712-5p (Qin et al., 2020). KLF4, an evolutionarily conserved zinc-finger-containing transcription factor, is thought to induce M2 and inhibit M1 macrophage polarization (Liao et al., 2011). A study found that the miR-375/KLF4 pathway plays a dominant role in macrophage polarization and autophagy, and tanshinone IIA could activate KLF4 by inhibiting miR-375, leading to enhanced autophagy as well as M2 polarization of macrophages (Chen et al., 2019a). Tropomyosin 1 (TPM1), as a target gene for miR-21-5p (Baker, 2011), is involved in the formation, stabilization and regulation of cytoskeletal actin fibers (Gunning et al., 2015). It was found that tanshinone IIA could downregulate miR-21-5p and then target TPM1, which helps to inhibit the proliferation and migration of VSMCs (Jia et al., 2019).

Salvianolic acid B, a water-soluble compound extracted from *S. miltiorrhiza* Bunge, has been used to treat cardiovascular diseases for hundreds of years. MiR-146a is involved in the regulation of cell proliferation, migration, differentiation, and apoptosis (Cheng et al., 2013). A study has found that salvianolic acid B can inhibit angiotensin II-induced VSMCs proliferation and improve carotid artery ligation-induced neointimal hyperplasia by downregulating miR-146a (Zhao et al., 2019).

Tanshinol is also an active ingredient isolated from *S. miltiorrhiza* Bunge which has the effect of protecting vascular

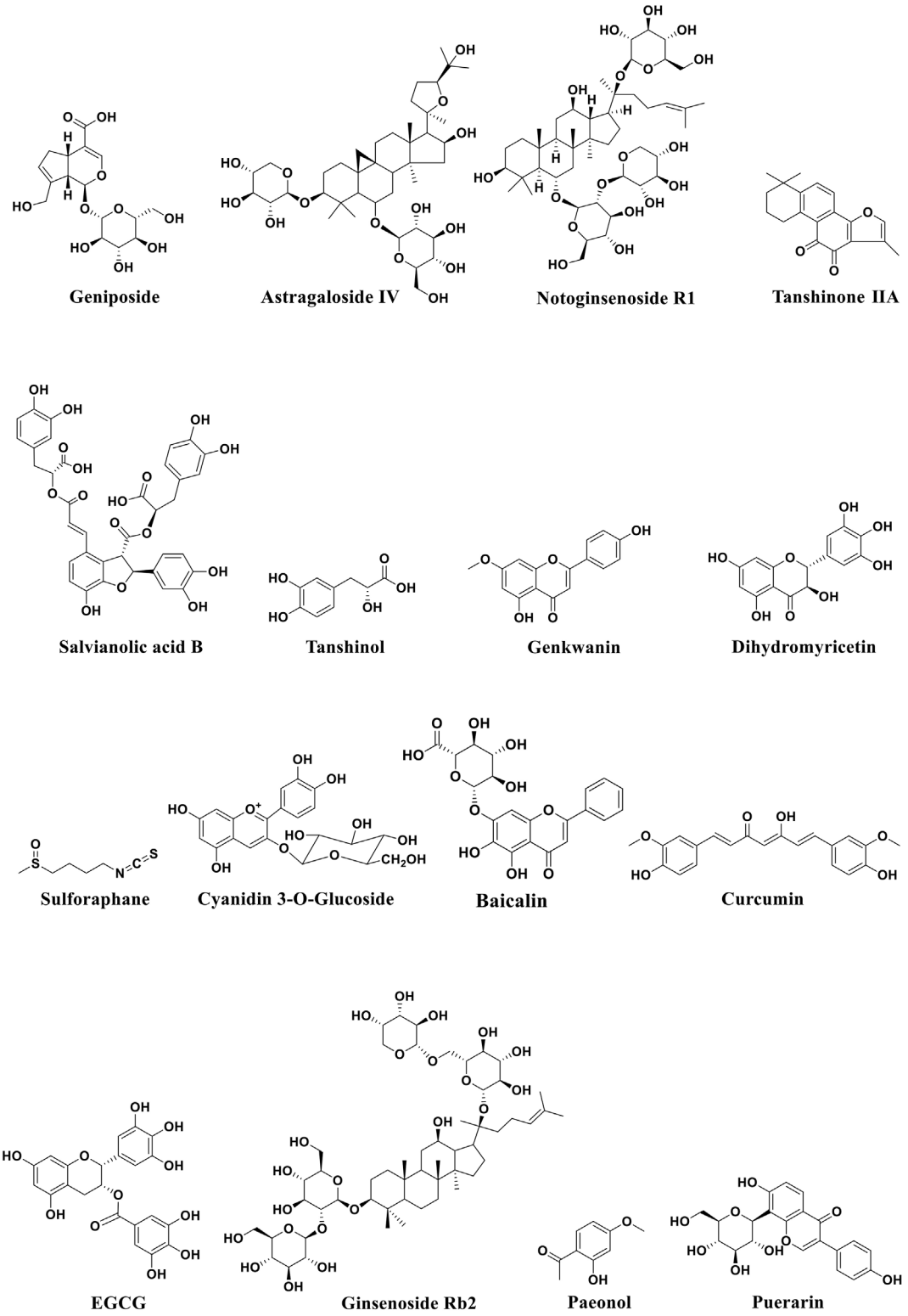


FIGURE 5
Structural formula of active monomers of traditional Chinese medicine that exhibit anti-atherosclerotic activities.

endothelium and reducing atherosclerosis (Song et al., 2014). MiR-26a has been proved to have anti-apoptotic effect on endothelial cells (Zhang et al., 2015). A study found that tanshinol inhibits apoptosis of endothelial cells and reduces atherosclerotic lesions via decreasing lncRNA TUG1 and increasing miR-26a in endothelial cells (Chen et al., 2016).

3.5 Genkwanin

Genkwanin is one of the major non-glycosylated flavonoids extracted from *Daphne genkwa* Siebold & Zucc. (Bao et al., 2019). MKP-1 is a key negative regulator of macrophage signaling in response to inflammatory stimulis and is responsible for shutting down the production of pro-inflammatory cytokines (Chen et al., 2002; Chi et al., 2006). Genkwanin was proved to potently decrease the production of proinflammatory mediators through downregulating miR-101 and increasing MKP-1 (Gao et al., 2014).

3.6 Dihydromyricetin

Dihydromyricetin, a bioactive flavonoid isolated from *Ampelopsis cantoniensis* var. *grossedentata* Hand. -Mazz. and *Ziziphus jujuba* Mill., has been found to have a wide range of pharmacological activities, such as anti-inflammatory (Sun et al., 2021), analgesic (Guan et al., 2019), anti-tumor (Chen et al., 2020) and hepatoprotective effects (Silva et al., 2020). Nitric oxide (NO), produced by endothelial nitric oxide synthase (eNOS), plays a key role in maintaining endothelial function, and impaired NO biosynthesis is a hallmark of atherosclerosis (Tousoulis et al., 2012; Cyr et al., 2020). There is evidence that overexpression of dimethylarginine dimethylaminohydrolase-1 (DDAH1) increases NO production through an asymmetric dimethylarginine (ADMA) manner (Pope et al., 2009). Studies suggested that dihydromyricetin treatment inhibits atherosclerotic lesion formation by increasing NO production by endothelial cells. MiR-21 expression can be reduced by dihydromyricetin in endothelial cells, which increases DDAH1 and reduces ADMA levels (Yang et al., 2018; Yang et al., 2020). Taken together, dihydromyricetin activates endothelial DDAH1/ADMA/eNOS/NO pathway by reducing miR-21, which relieves the pathogenesis of atherosclerosis.

3.7 Sulforaphane

Sulforaphane is an isothiocyanate, which is produced by the conversion of glucoraphanin through the myrosinase (Vanduchova et al., 2019). Sulforaphane, a potent antioxidant, is primarily found in several Brassicaceae vegetables, such as broccoli, cauliflower, cabbage, and Brussels sprouts. Sulforaphane has often been shown to protect cells from oxidative stress in cardiomyocytes and neural cells (Guerrero-Beltran et al., 2012). The nuclear factor erythroid-2-related factor 2 (Nrf2), a basic leucine zipper transcription factor that serves as a defense mechanism against oxidative stress, has been shown to be activated by sulforaphane (Bai et al., 2015; Houghton et al., 2016). SIRT1 is a potential target gene of miR-34a (Yamakuchi et al., 2008) and the role of the miR-34a/SIRT1 axis in oxidative stress-induced cellular damage has been demonstrated (Guo et al., 2017). Sulforaphane was found to protect endothelial cells from oxidative stress by regulating the miR-34a/SIRT1 axis through upregulation of Nrf2 (Li et al., 2021c). In addition, a study found that sulforaphane can reduce lipopolysaccharide-induced cell damage and oxidative stress by inhibiting miR-155 in microglia (Eren et al., 2018). MiR-155 was proved to aggravate the carotid atherosclerotic lesion through induction of endothelial cell apoptosis and activation of inflammasome in macrophages (Yin et al., 2019b). Therefore, it is possible that sulforaphane may limit the formation of atherosclerotic lesions by inhibiting miR-155, but clearly, more studies are needed to confirm this hypothesis.

3.8 Cyanidin-3-O-glucoside

Anthocyanins are abundant natural water-soluble pigments, which are relatively rich in the skin of *Glycine max* (L.) Merr. These compounds have been shown to exert antioxidant and anti-inflammatory properties (Zhang et al., 2020a). Cyanidin-3-O-glucoside is one of the most abundant anthocyanins in nature. A study found that cyanidin-3-O-glucoside treatment not only suppresses blood lipids, but also improves endothelial cell function in a rabbit atherosclerotic model. Mechanistically, these effects are due to decreased expression of miR-204-5p, which leads to the increased expression of SIRT1 and enhanced endothelial cell function (Wang et al., 2020c).

3.9 Baicalin

Baicalin, one of the flavonoid compounds, is the main active component of traditional Chinese medicine *Scutellaria baicalensis* Georgi (Li et al., 2009). It has been shown that baicalin can alleviate the development of atherosclerosis through its anti-adipogenic, anti-inflammatory and antioxidant effects (Wu et al., 2018). The expression of miR-126 was found to be reduced in the peripheral blood of atherosclerotic patients (Jiang et al., 2014). High mobility group box 1 protein (HMGB1) is an essential facilitator of atherosclerosis by enhancing inflammation (Boteanu et al., 2017). It has been found that baicalin induces the upregulation of miR-126-5p and the downregulation of HMGB1, inhibiting ox-LDL-induced proliferation and migration of VSMCs (Chen et al., 2019b).

3.10 Curcumin

Curcumin is the main active ingredient of *Curcuma longa* L. and is mainly extracted from dried powdered turmeric. There is evidence that curcumin can modulate the inflammatory response and alleviate inflammatory diseases like atherosclerosis (Hasan et al., 2014; Chen et al., 2015). Studies found that the activated miR-126-3p from endothelial cells and VSMCs plays a key role in reducing vascular calcification (Zeng et al., 2021) and curcumin upregulates miR-126-3p expression (Li et al., 2021b). Therefore, we infer that miR-126-3p may be one of the targets of curcumin in the treatment of atherosclerosis. LncRNA MIAT has been shown to aggravate the atherosclerotic damage through the activation of PI3K/Akt signaling pathway (Sun et al., 2019). A study found that reduced expression of MIAT contributes to the protective effect of curcumin on atherosclerosis. MIAT regulates miR-124 by interacting with enhancer of zeste homolog 2 (EZH2), thereby relieving inflammation in endothelial cells (Ouyang et al., 2022). In addition, curcumin markedly suppresses miR-125a-5p and

upregulates SIRT6 in macrophages, thereby regulating the ABCA1 expression and promoting cholesterol efflux of macrophages (Tan et al., 2021).

3.11 Epigallocatechin gallate

Epigallocatechin gallate (EGCG) is the most abundant catechin in green tea. EGCG has been shown to has various pharmacological effects including the anti-atherosclerotic effect, which is primarily achieved by promoting intracellular cholesterol efflux in macrophages (Jiang et al., 2012). Recent studies showed that miR-33a is an upstream regulator of ABCA1 (Wijesekara et al., 2012) and EGCG exerts anti-atherosclerotic effect by reducing miR-33a, thereby upregulating ABCA1 and promoting the efflux of cholesterol in macrophages (Yang et al., 2016).

3.12 Ginsenoside Rb2

Ginsenoside Rb2, extracted from *Panax ginseng* C.A. Mey., is a commonly used traditional Chinese medicine with antioxidant (Huang et al., 2014), anti-inflammatory (Huang et al., 2017) and anti-apoptotic activities (Gao et al., 2015). In macrophages, ginsenoside Rb2 has been found to exert anti-inflammatory effects by upregulating the expression of an ω-3 fatty acid receptor GPR120 (Huang et al., 2017). A recent study showed that ginsenoside Rb2 can also inhibit endothelial senescence and inflammation. Mechanistically, ginsenoside Rb2 has a specific binding affinity for miR-216a and further attenuates miR-216a-induced inflammatory processes and aging states through the Smad3/IκBα signaling pathway (Chen et al., 2021b).

3.13 Paeonol

Paeonol is one of the main active compounds in Tree Peony Bark, which has been found to have anti-inflammatory, anti-thrombotic and antioxidant properties (Fu et al., 2012a; Bao et al., 2013). Paeonol could increase the expression of miR-223 in macrophage-derived exosomes, and after the uptaking of exosomes by endothelial cells, the STAT3 signaling and the related inflammatory response in endothelial cells can be attenuated (Liu et al., 2018). Another study also found similarly protective results of paeonol on endothelial cells in hyperlipidemia-induced atherosclerosis, which is also attributed to cellular uptake of exosomal miR-223 (Shi et al., 2020). Additionally, paeonol also promotes miR-126 expression to inhibit monocyte adhesion to endothelial cells and block the activation of the PI3K/Akt/NF-κB signaling pathway (Yuan et al., 2016). Moreover, miR-21 and its target PTEN also contribute to the protective effects of paeonol on ox-LDL-induced endothelial injury (Liu et al., 2014). MiR-338-3p was proved to be increased in atherosclerotic lesions, and paeonol treatment could downregulates the expression of miR-338-3p and upregulates the expression of Tet methylcytosine dioxygenase 2 (TET2), thereby relieving ox-LDL-induced endothelial cell damage (Yin et al., 2019a; Yu et al., 2020b). Paeonol can also weaken ox-LDL-induced endothelial autophagy through regualting miR-30a/beclin-1 signaling (Li et al., 2018a). Overall, these studies indicate that paeonol has strong endothelial protective effects, which is associated with regulation of various miRNAs and their targets.

3.14 Puerarin

Pueraria lobata is the dried roots of legumes *P. lobata* (Willd.) Ohwi and *Pueraria thunbergiana* (Siebold & Zucc.) Benth. It is clinically used in the treatment of cardiovascular and cerebrovascular diseases (Wang et al., 2020a). Puerarin, an active monomer in Pueraria lobata, was reported to inhibit the proliferation and inflammation of VSMCs in atherosclerosis by reducing the expression of miR-29b-3p, thereby increasing the expression of insulin-like growth factor 1 (IGF1) (Li et al., 2023). Therefore, puerarin may have a beneficial effect in the treatment of atherosclerosis by regulating miRNA.

4 Conclusion and prospects

Atherosclerosis is a major cause of coronary heart disease, cerebral infarction, and some peripheral vascular diseases (Figure 1). With the improvement of living standards, the incidence and mortality of atherosclerosis-induced cardiovascular diseases have increased rapidly in recent years. During the development of atherosclerosis, abnormal expressions of ncRNAs affect the physiological functions of endothelial cells, macrophages, and VSMCs by regulating related signaling pathways or specific proteins. China has a long history of using herbal medicine to treat cardiovascular diseases and the anti-atherosclerotic effects of several herbal medicine are also demonstrated in animal experiments and human studies. The traditional Chinese medicine monomers have recently attracted more attention in the treatment of diseases because they have certain molecular structures, predicted pharmacological effects, less drug-drug interactions, and clear mechanisms of action. Many active monomers derived from traditional Chinese medicines have been evaluated *in vivo* and *in vitro* to ameliorate the development of atherosclerosis by targeting ncRNAs. This article reviews 16 active monomers in traditional Chinese medicine that can improve the development of atherosclerosis by targeting ncRNAs in endothelial cells, macrophages, and VSMCs (Table 1; Figures 2–4). Their structures are shown in Figure 5. Besides monomeric Chinese herbal extracts, Chinese herbal formulas and decoctions have also been proved to treat atherosclerosis by targeting ncRNAs. For example, Tongxinluo Capsule inhibits vascular inflammation and neointimal hyperplasia by inhibiting the expression of miR-155, thereby blocking the feedback loop between miR-155 and TNF-α (Zhang et al., 2014). Alismatis rhizoma decoction, a classic traditional Chinese Medicinal formula used for the treatment of cardiovascular and cerebrovascular diseases, can inhibit the expression of ERK1/2 and miR-17~92a to inhibit ox-LDL-stimulated VSMCs proliferation (Shen et al., 2020). Among the ncRNAs regulated by active monomers of traditional Chinese medicine, miRNAs are the most studied. However, whether traditional Chinese medicine can exert functions via

regulating lncRNAs, circRNAs or other ncRNAs is not well studied and requires more research.

NcRNAs are the most abundant transcripts in cells. In addition to seaching ncRNAs from published papers, we can identify or screen new ncRNAs in the following ways: firsty, we can utilize publicly available genomic and transcriptomic databases, such as Ensembl, NCBI, and UCSC to identify regions of the genome that are transcribed but not coding for proteins, indicating potential ncRNAs; additionally, high-throughput sequencing like RNA-Seq can be used to identify novel transcripts, including potential ncRNAs; furthermore, we can also compare the genomic sequences across various species in order to find conserved non-coding regions, which may potentially serve as ncRNAs; besides, machine learning algorithms based on sequence features and structural properties of ncRNAs can also be used to predict potential novel ncRNAs. After discovering new ncRNAs, techniques like CRISPR/Cas9, RNA interference, qRT-PCR, Northern blotting, *in situ* hybridization and other functional assays can be used to identify the specific biological functions of the ncRNAs. It can be expected that future studies will find more and more ncRNAs that related to atherosclerosis and these ncRNAs can be used as drug targets for development of anti-atherosclerotic drugs.

Over the past decades, substantial effort has been made towards the clinical application of RNA-based therapeutics, such as small interfering RNAs and antisense oligonucleotides. However, since the hurdle of immunogenicity, specificity, and delivery, some studies demonstrated limited efficacy or toxicity of ncRNAs-based therapies. Therefore, traditional Chinese medicine may become alternative drugs by targeting ncRNAs to treat atherosclerosis. It is worth noting that most studies suggest that traditional Chinese medicine treats atherosclerosis by targeting a specific ncRNA. However, the mechanism of ceRNA suggests that ncRNAs may have complex interactions in cells. What's more, a ncRNA may also have multiple targets. Therefore, we should further explore the anti-atherosclerotic mechanisms and clinical safety of these traditional Chinese medicine in more detail. It is hoped that by studying the regulation of ncRNAs by traditional Chinese medicine, it will provide theoretical support for the future research and clinical application of traditional Chinese medicine for treatment of atherosclerosis.

While many traditional Chinese medicines have therapeutic effects on atherosclerosis, some research has also identified potential side effects of certain Chinese herbs that can exacerbate atherosclerosis. For example, a moderate dosage of marijuana proves highly efficient in alleviating chronic pain (Carter et al., 2015), but marijuana can also cause cardiovascular side effects, such as endothelial dysfunction and atherosclerosis (Feng et al., 2022). Proanthocyanidin A1 can promotes the production of platelets to ameliorate chemotherapy-induced thrombocytopenia (Wang et al., 2022) and TMEA, a polyphenol in *Sanguisorba officinalis* L., can facilitate megakaryocyte differentiation and platelet production (Li et al., 2021a). However, the increased platelets can raise the risk of blood clot formation in patients with atherosclerosis (Barrett et al., 2019). Therefore, when patients have concurrent atherosclerosis, the use of these Chinese herbal medicines should be avoided. Furthermore, studying the ncRNAs that may mediate these effects is of significant importance, but this field is still lacking in research and requires further investigation.

Author contributions

GL: Conceptualization, Data curation, Methodology, Writing–original draft. LT: Writing–review and editing. XZ: Data curation, Writing–review and editing. MW: Software, Writing–review and editing. ZZ: Data curation, Writing–review and editing. JZ: Validation, Writing–review and editing. HG: Data curation, Writing–review and editing. ML: Writing–review and editing. WQ: Funding acquisition, Supervision, Writing–review and editing, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

ABCA1	ATP-binding cassette transporter A1
ADMA	Asymmetric dimethylarginine
ApoE$^{-/-}$ mice	Apolipoprotein e-knockout mice
ceRNA	Competitive endogenous RNA
circRNAs	Circular RNAs
CLIC4	Chloride intracellular channel 4
DDAH1	Dimethylarginine dimethylaminohydrolase-1
DUSP5	Dual-specificity phosphatase 5
EGCG	Epigallocatechin gallate
eNOS	Endothelial nitric oxide synthase
EZH2	Enhancer of zeste homolog 2
HAEC	Human aortic endothelial cells
HASMCs	Human airway smooth muscle cells
HA-VSMCs	Human aortic vascular smooth muscle cells
HMGA2	High mobility group AT-hook 2
HMGB1	High mobility group box 1 protein
HMG-CoA	Hydroxymethylglutaryl-CoA
HUVECs	Human umbilical vein endothelial cells
hVSMCs	Human vascular smooth muscle cells
IGF1	Insulin-like growth factor 1
KLF4	Krüppel-like factor 4
lncRNAs	Long non-coding RNAs
MCP-1	Monocyte chemoattractant protein 1
miRNAs	MicroRNAs
MKP-1	Mitogen-activated protein kinase phosphatase-1
MyD88	Myeloid differentiation primary response gene 88
ncRNAs	Non-coding RNAs
NF-κB	Nuclear factor-κB
NO	Nitric oxide
Nrf2	Nuclear factor erythroid-2-related factor 2
ox-LDL	Oxidized low-density lipoprotein
p53	Tumor protein 53
PCSK9	Proprotein convertase subtilisin kexin 9
PTEN	Phosphatase and tensin homolog
RAECs	Rat aortic endothelial cells
RAW264.7	RAW 264.7 mouse leukemia macrophage cell line
SD rats	Sprague-dawley rats
SIRT1	Sirtuin 1
SIRT6	Sirtuin 6
Smad3	Sma- and mad-related protein 3
SOD	Superoxide dismutase
STAT3	Signal transducer and activator of transcription 3
TET2	Tet methylcytosine dioxygenase 2
THP-1	Human acute monocytic leukemia cell line
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor-α
TPM1	Tropomyosin 1
TRAF6	TNF receptor-associated factor 6
VCAM-1	Vascular cell adhesion molecule-1
VECs	Vascular endothelial cells
VLDLR	Very low-density lipoprotein receptor
VSMCs	Vascular smooth muscle cells

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EDITED BY
Yusof Kamisah,
Faculty of Medicine Universiti Kebangaan Malaysia, Malaysia

REVIEWED BY
Masashi Tawa,
Osaka Medical and Pharmaceutical University, Japan
Gizem Kayki Mutlu,
Ankara University, Türkiye

*CORRESPONDENCE
Yuan Bian,
bianyuan567@126.com
Lizhu Han,
yummylia@163.com

†The author shares first authorship

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Decoding signaling mechanisms: unraveling the targets of guanylate cyclase agonists in cardiovascular and digestive diseases

Qinan Yin[1,2†], Xingyue Zheng[1,2†], Yujie Song[1,2†], Liuyun Wu[1,2], Lian Li[1,2], Rongsheng Tong[1,2], Lizhu Han[1,2*] and Yuan Bian[1,2*]

[1]Department of Pharmacy, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China, [2]Personalized Drug Therapy Key Laboratory of Sichuan Province, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China

Soluble guanylate cyclase agonists and guanylate cyclase C agonists are two popular drugs for diseases of the cardiovascular system and digestive systems. The common denominator in these conditions is the potential therapeutic target of guanylate cyclase. Thanks to in-depth explorations of their underlying signaling mechanisms, the targets of these drugs are becoming clearer. This review explains the recent research progress regarding potential drugs in this class by introducing representative drugs and current findings on them.

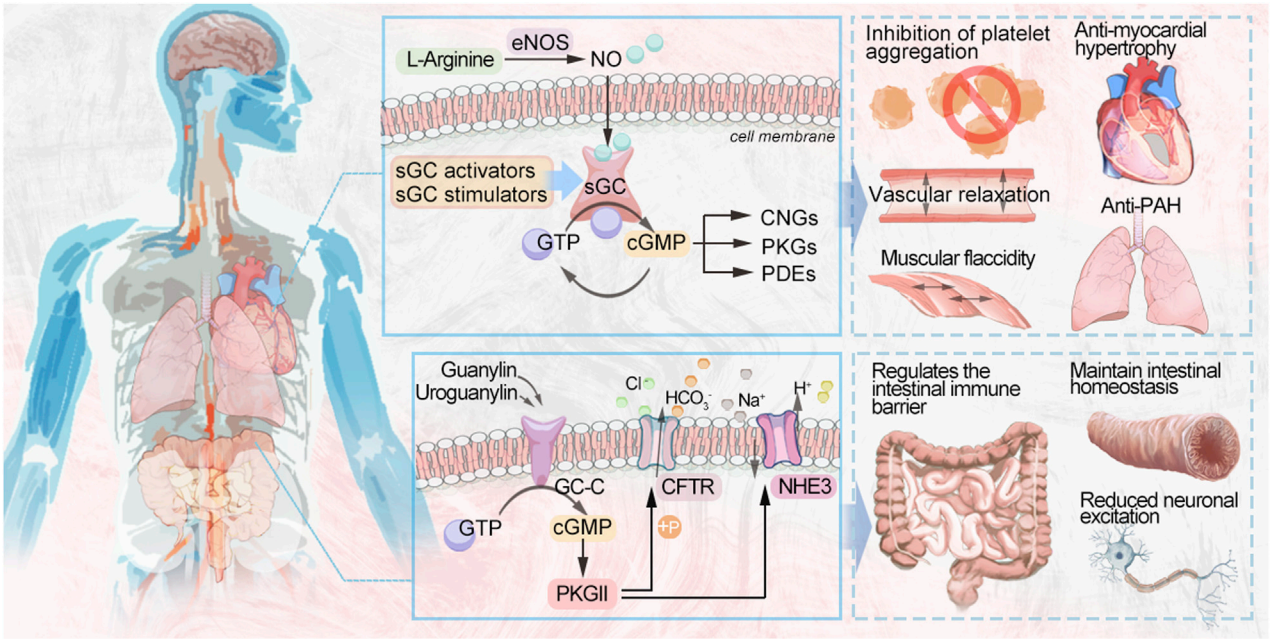
KEYWORDS

soluble guanylate cyclase stimulators, soluble guanylate cyclase activators, guanylate cyclase-c agonists, mechanism, signaling pathway

Introduction

Guanylate cyclase (GC) is a protease that catalyzes the conversion of guanylate triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). Based on the properties of the distributed form of the enzyme, it can be divided into two categories: membrane-bound guanylate cyclase and soluble guanylate cyclase (sGC). Both are widely distributed in the human body, including the lung, brain, kidney, blood vessels, and other tissues (Garbers, 1990). The agonists of membrane-bound guanylate cyclase are peptides (natriuretic peptides A, B, and C), and the agonists of sGC are gaseous mediators (NO and CO) (Grzesk et al., 2023).

sGC is a heterodimer with an α-subunit and β-subunit, of which the latter contains the heme-nitric oxide/oxygen (H-NOX) domain (Argyriou et al., 2021). As a result of its affinity for nitric oxide (NO), this enzyme has also been called an NO-sensitive guanosyl cyclase. NO binding to sGC heme increases GTP cyclase activity, resulting in the production of cGMP, which regulates multiple signaling pathways in cells (Stasch et al., 2011). The cardiovascular, pulmonary, and neurological systems, as well as organs like the kidney, brain, and liver, are highly dependent on NO-sGC-cGMP regulation. Regarding its role in regulation, fibroblasts, cardiomyocytes, platelets, neurons, and immune cells are also affected by cGMP, and it controls fibrosis, the inflammatory response, and neurotransmission (Derbyshire and Marletta, 2009; Grzesk and Nowaczyk, 2021; Nowaczyk et al., 2021).



GRAPHICAL ABSTRACT
Possible therapeutic indications for sGC agonists and GC-C agonists.

Guanylate cyclase C (GC-C) is a member of the membrane-bound GC family. It consists of an extracellular domain (ECD) and an intracellular domain, connected by a single strand across the membrane region. In the cell, it synthesizes cGMP and regulates an array of intracellular physiological functions (Hasegawa and Shimonishi, 2005). Reviewing recent progress in research on sGC agonists and GC-C agonists, along with their mechanisms, is the purpose of this paper.

Soluble guanylate cyclase agonists

sGC is a heterodimeric enzyme with a prosthetic heme group (Archer, 2013). Many physiological functions depend on NO signaling as their primary sensor. sGC binding of NO results in a significant increase in sGC enzyme activity. Additionally, cGMP is produced in a complex with its ligand, NO. The cascades of NO-driven signaling are amplified by sGC (Kang et al., 2019). Only the sGC receptor responds to gaseous NO (Schmidt et al., 2003). cGMPs produced by sGC help regulate the cardiovascular, neuronal, and digestive systems. In pharmacology and therapeutics, improving sGC activity and preventing or reversing inactivation are important goals (Stuehr et al., 2021). There are two forms of sGC in the body: those that respond to NO and those that do not. In brief, sGC contains a heme moiety, which is either ferrous (reduced sGC) or ferric (oxidized sGC). In these forms, the sGC stimulator can only target the reduced, heme-containing sGC, whereas the sGC activator binds to the oxidized or heme-free sGC, resulting in increased production of cGMPs (Dai and Stuehr, 2022). Although sGC activators stimulate heme-containing enzymes independently of NO, NO enhances their activity; even when oxidative stress occurs, cGMP is released by sGC activators (Dai and Stuehr, 2022). These novel pharmacological principles of sGC stimulation and activation appear to have very broad therapeutic potential. Signaling pathways such as NO-sGC-cGMP play an important role in cellular homeostatic maintenance and physiology (Sandner et al., 2021a). The current progress in understanding sGC agonists is summarized in Tables 1, 2.

The NO-sGC-cGMP signaling pathway

Three different nitric oxide synthases (NOS) are involved in reducing L-arginine to citrulline endogenously (Figure 1): endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS), and induced nitric oxide synthase (iNOS) (Sandner et al., 2018). During vascular NO-sGC-cGMP signaling, L-arginine is converted to NO in the endothelial monolayer by endothelial nitric oxide synthase (eNOS) and diffuses into the vascular lumen and the vessel wall, thereby activating sGC. Heme-dependent sGC stimulators and nonheme-dependent sGC activators increase cellular cGMP concentrations by directly activating sGC, leading to vascular relaxation and inhibiting platelet aggregation (Evora et al., 2012). NO produced through endothelial cells plays an important biological role. cGMP is synthesized by NO by binding to the active heme-containing sGC in vascular smooth muscle cells. Smooth muscle sGCs are NO signaling targets. The binding of NO to sGC leads to the conversion of GTP to cGMP. The resulting cGMP is hydrolyzed after binding to one of three types of cGMP effector proteins, including gated cation channels, the protein kinases (PKGs) that are dependent on cGMP, and the phosphodiesterases (PDEs) that are regulated by it (Feil et al., 2022).

TABLE 1 Current progress on soluble guanylate cyclase stimulators.

Drug	Trial name	Design	Population	Dose	Trial ID	Endpoint	Safety outcome	Stage of development	Conclusion	Reference
Riociguat	PATENT-1	Randomized, Double-blind, Placebo-controlled, Multicenter, Multinational Trial	Patients with PAH	1–2.5 mg tid	NCT00810693	Change in 6MWD	AEs, SAEs and deaths	Completed	Riociguat significantly improved exercise capacity and secondary efficacy end points in patients with PAH	Toxvig et al. (2019)
Riociguat	RESPITE	Open-label, International, Multicenter, Single-arm, Uncontrolled, Phase IIIb Trial	Patients with CTEPH	1–2.5 mg tid	NCT02007629	Change in 6MWD	AEs	Completed	Patients with PAH may benefit from switching from PDE5i to riociguat	Barnikel et al. (2022)
Riociguat	RioSAPH	Double Blind, Placebo Controlled Trial	Patients with SAPH	2.5 mg tid	NCT02625558	Time until clinical worsening	AEs	Unknown	Riociguat was effective in preventing clinical worsening and improving exercise capacity in patients with SAPH.	Baughman et al. (2022)
Riociguat	ISE-IIP	Randomized, Double-blind, Placebo-controlled Phase II Trial	Patients with IIP or PAH	0.5–2.5 mg tid	NCT02138825	Change in PVR at week 26	AEs and SAEs	Terminated	Riociguat should not be used in patients with PH-IIP due to increased serious adverse events and mortality and an unfavorable risk-benefit profile	Nathan et al. (2019)
Riociguat	PATENT-CHILD	Open-label, Individual Trial	Children with PAH	0.5–2.5 mg tid	NCT02562235	Change in 6MWD to Week 16	AEs and SAEs	Active, not recruiting	A suitable riociguat dosing strategy for pediatric patients with PAH have an acceptable safety profile with potential efficacy signals	Garcia et al. (2022)
Riociguat	RISE-SSc	Randomized, Double-Blind, Placebo-Controlled Phase II Trial	Patients with dcSSc	0.5–2.5 mg tid	NCT02283762	Change in mRSS	AEs and SAEs	Completed	Riociguat did not significantly benefit mRSS versus placebo	Khanna et al. (2020)
Vericiguat	SOCRATES-REDUCED	Randomized Parallel-group, Placebo-controlled, Double-blind, Multicenter Phase II Trial	Patients with HF	1.25 mg, 2.5 mg, 5 mg or 10 mg, qd	NCT01951625	Change in NT-proBNP	Changes in LVEF; LVEDV; LVESV	Completed	Vericiguat had no significant effect on NT-proBNP levels in patients with worsening chronic HF and reduced LVEF but was well-tolerated	Gheorghiade et al. (2015)
Vericiguat	VICTORIA	Randomized Parallel-Group, Placebo-Controlled, Double-Blind, Multi-Center Pivotal Phase III Trial	Patients with HF	2.5, 5.0, or 10.0 mg, qd	NCT02861534	Time to Composite Endpoint of Cardiovascular Death or Heart Failure Hospitalization	Symptomatic hypotension and syncope	Completed	Compared to placebo, vericiguat significantly reduced the incidence of the composite endpoint	Armstrong et al. (2020a)
Vericiguat	SOCRATES-PRESERVED	Randomized Parallel-group, Placebo-controlled, Double-blind,	Patients with HFpEF	fixed-dose (1.25 mg or 2.5 mg) and	NCT01951638	Change in NT-proBNP and LAV From Baseline	AEs and SAEs	Completed	Vericiguat was well tolerated and did not alter NT-proBNP and LAV at	Pieske et al. (2017)

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TABLE 1 (*Continued*) Current progress on soluble guanylate cyclase stimulators.

Drug	Trial name	Design	Population	Dose	Trial ID	Endpoint	Safety outcome	Stage of development	Conclusion	Reference
		Multicenter Dose Finding Phase II Trial		uptitrated to 5 mg or 10 mg, qd					12 weeks compared to placebo, but was associated with improved quality of life in patients with HFpEF	
Vericiguat	VITALITY	Multicenter, randomized, double-blind, placebo-controlled phase IIb trial	Patients with HFpEF	uptitrated to 10 mg or 15 mg, qd	NCT03547583	Change in KCCQ physical limitation score and 6MWD from baseline	TEAEs	Completed	No significant change in KCCQ or 6MWD compared to placebo at 24 weeks	Armstrong et al. (2020b)
Vericiguat	VENICE	Multicenter, Randomized, Placebo-controlled, Double-blind Group Comparison Trial	Patients with CCSs	2.5, 5, or 10 mg, qd	NCT02617550	Measurements of the hemodynamic profile	AEs and SAEs	Completed	The combination of Vericiguat with nitroglycerin administered to patients with CCSs was well tolerated	Boettcher et al. (2022)
Praliciguat	CAPACITY HFpEF	Multicenter, Randomized, Double-blind, Placebo-controlled, Phase 2 Trial	Patients with HFpEF	10, 20, or 40 mg daily	NCT03254485	Change in peak VO2	TEAEs	Completed	These findings do not support the use of praliciguat in patients with HFpEF.	Udelson et al. (2020)
Praliciguat	/	Phase IIA, double-blind, placebo-controlled trial	Patients with 2 diabetes and hypertension	20 mg bid or 40 mg qd	NCT03091920	ABPM and HOMA-IR	TEAEs	Completed	Praliciguat was well tolerated and showed positive trends in metabolic and BP variables	Hanrahan et al. (2020a)
Praliciguat	/	Randomized, Double-Blind, Placebo-Controlled, Phase 2 Trial	Patients with type 2 Diabetes Mellitus combined with Diabetic Nephropathy	20 mg or 40 mg qd	NCT03217591	Change in urine albumin–creatinine ratio	TEAEs	Completed	Praliciguat treatment did not significantly reduce albuminuria compared with placebo	Hanrahan et al. (2020a)
Olinciguat	/	Multicenter, Randomized, Double-blind, Placebo-controlled, Parallel-group, Single-dose, Phase 2a Study	Patients with Achalasia	Single 5 mg dose	NCT02931565	Change in BFT	TEAEs、SAEs and ADOs	Terminated	/	/
Olinciguat	STRONG SCD	Randomized, Placebo-controlled, Phase 2 Study	Patients with Sickle Cell Disease		NCT03285178	/	TEAEs and SAEs	Completed	/	/

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TABLE 2 Current progress in soluble guanylate cyclase activators.

Drug	Trial name	Design	Population	Dose	Trial ID	Endpoint	Safety outcome	Stage of development	Conclusion	Reference
Cinaciguat	/	Placebo Controlled, Randomized, Double-blind, Multicenter, Multinational Phase IIb Study	Patients with ADHF	Uptitration from 100–600 μg/g, over maximum 48 h	NCT00559650	Change in PCWP	AEs 、SAEs and TEAEs	Terminated	Cinaciguat unloaded the heart in patients with ADHF. High doses were associated with hypotension	Erdmann et al. (2013)
Cinaciguat	COMPOSE 1	Placebo Controlled, Randomized, Double-Blind, Multicenter, Phase IIb Study	Patients with ADHF	50 μg/h; 100 μg/h or 150 μg/h during 48 h	NCT01065077	Change in PCWP or LOCF	Change in heart rate; SBP; frequency of SAEs and TEAEs	Terminated	It is doubtful that further studies with intravenous cinaciguat would prove beneficial in ADHF patients	Gheorghiade et al. (2012)
Cinaciguat	COMPOSE 2	Placebo Controlled, Randomized, Double-Blind, Multicenter, Phase IIb Study	Patients with ADHF	10 μg/h and 25 μg/h during 48 h	NCT01067859	Change in PCWP or LOCF	Change in heart rate; SBP; frequency of SAEs and TEAEs	Terminated	No statistical analysis was performed	Gheorghiade et al. (2012)
Cinaciguat	COMPOSEE EARLY	placebo controlled, double-blind and randomized study	Patients with ADHF	50 μg/h; 100 μg/h or 150 μg/h during 48 h	NCT01064037	The change in dyspnea assessed using a VAS or LOCF	Change in heart rate; SBP; frequency of SAEs and TEAEs	Terminated	No significant clinical benefit of cinaciguat	Gheorghiade et al. (2012)
Ataciguat	/	Phase Ib Randomized, Placebo-controlled, Double-blinded Study	Patients with Moderate CAVS	50 mg, 100 mg, or 200 mg qd	NCT02049203	The change in blood pressure following the transition from sitting to standing	Number of patients experiencing orthostatic hypotension	Completed	/	/
Ataciguat	SERENEATI	Randomized, Double-blind, Placebo-controlled, Crossover Study	patients with neuropathic pain	200 mg qd	NCT00799656	Change in average daily pain intensity; change in NPSI	Rescue medication intake	Completed	/	/
Ataciguat	ACCELA	Randomized, Double-blind, Placebo-controlled, Parallel Group Trial	Patients with PAD	/	NCT00443287	change in ICD	AEs	Completed	/	/
Ataciguat	CAVS	Phase II Randomized, Placebo-Controlled, Double-Blinded Study	Patients with AVC	200 mg qd	NCT02481258	Changes in Aortic Valve Calcium Levels	/	Completed	/	/
MGV354	/	Randomized, Double-masked, Placebo-Controlled, Safety, Tolerability and Early Efficacy Study	Patients with Ocular Hypertension or Glaucoma	3 μg per eye to 300 μg per eye	NCT02743780	Change in Diurnal IOP	AEs and TEAEs	Completed	Human glaucomatous trabecular meshwork may have levels of oxidized sGC that are too low to benefit from MGV354	Stacy et al. (2018)
Runcaciguat	CONCORD	Randomized, Double-blind, Placebo-controlled, Multicenter Study	Patients with CKD With Diabetes and/or Hypertension	/	NCT04507061	Mean change in UACR	TEAEs; number of subjects with early discontinuations	Completed	/	/
Runcaciguat	NEON-NPDR			Oral dose	NCT04722991	DRSS improvement	TEAEs		/	/

(Continued on following page)

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TABLE 2 (*Continued*) Current progress in soluble guanylate cyclase activators.

Drug	Trial name	Design	Population	Dose	Trial ID	Endpoint	Safety outcome	Stage of development	Conclusion	Reference
		Phase 2 Randomized, Placebo-controlled, Double-masked Proof-of-concept Study	Patients with NPDR					Active, not recruiting		
Mosliciguat	ATMOS	Nonrandomized Two Part Multicenter, Open-label, Single Dose Trial	Patients with PAH or CTEPH	up to a maximum dose of 4,000 μg	NCT03754660	reduction in PVR	TEAEs	Completed	/	/
BI-685509	/	Randomized, Double-blind, Placebo-controlled and Parallel Group Trial	Patients with CSPH	/	NCT05161481	Percentage change in HVPG	Decompensation events; CTCAE	Recruiting	/	Reiberger et al. (2023)
BI-685509	/	Randomized, Open-label and Parallel Group Trial	Patients with CSPH		NCT05282121	Percentage change in HVPG	Decompensation events; CTCAE	Recruiting	/	Reiberger et al. (2023)
BI-685509	/	Phase II, Randomized, Placebo-controlled, Double-blind, Parallel Group, Study	Patients with SSc	/	NCT05559580	Rate of decline in FVC	Time to treatment failure	Recruiting	/	/
BI-685509	/	Randomized, Double-blind (Within Dose Groups), Placebo Controlled and Parallel Group Trial	Patients with CKD	/	NCT04736628	Change in UACR	/	Active, not recruiting	/	/
BI-685509	/	Randomized, Double-blind, Placebo-controlled Trial	Patients with DKD	1 mg tid; 3 mg qd; 3 mg tid	NCT03165227	Change in log transformed UACR	AEs 、 SAEs	Completed	BI 685509 was generally well tolerated	Cherney et al. (2023)
BI-685509	/	Randomized, Double-blind (Within Dose Groups), Placebo Controlled and Parallel Group Trial	Patients with NPDR	/	NCT04736628	Change in log transformed UACR	/	Active, not recruiting	/	/
BI-685509		Randomized, Double-blind (Within Dose Groups), Placebo-controlled and Parallel Group Trial	Patients with DKD	/	NCT04750577	Change in log transformed UACR	/	Completed	/	/

PCWP, pulmonary capillary wedge pressure; AEs, adverse events; SAEs, serious adverse events; TEAEs, treatment-emergent adverse events; ADHF, acute decompensated heart failure; SBP, systolic blood pressure; VAS, visual analog scale; LOCF, last observation carried forward; CAVS, calcific aortic valve stenosis; NPSI, neuropathic pain symptom inventory; PAD, peripheral arterial disease; ICD, initial claudication distance; AVC, aortic valve calcification; CKD, chronic kidney disease; UACR, urinary albumin-to-creatinine ratio; NPDR, nonproliferative diabetic retinopathy; DRSS, diabetic retinopathy severity scale; PAH, pulmonary arterial hypertension; CTEPH, chronic thromboembolic pulmonary hypertension; CSPH, clinically significant portal hypertension; HVPG, hepatic venous pressure gradient; CTCAE, common terminology criteria for adverse events; SSc, systemic sclerosis; FVC, forced vital capacity; DKD, diabetic kidney disease.

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Disruption of the NO-sGC-cGMP signaling pathway is central to the pathogenesis of pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH), in which endothelial dysfunction leads to impaired NO synthesis. The progression of PAH and CTEPH is also associated with low NO production. The sGC stimulators have a dual role in that they directly stimulate the native form of the enzyme, making it more sensitive to endogenous NO and increasing sGC activity regardless of NO and cGMP levels, leading to an increase in cGMP. (Benza et al., 2019). The vasodilation effect of cGMP in the pulmonary circulation is mediated by a variety of subcellular mechanisms, one of which is the activation of cGMP-dependent protein kinase, phosphorylating calcium-sensitive potassium channels (BKCa), leading to potential hyperpolarization of the pulmonary artery smooth muscle membrane and inhibiting calcium inflow through L-type Ca^{2+} channels (LTCCs). sGC is a redox-sensitive enzyme that is activated by hydrogen peroxide, causing pulmonary artery blood vessels to dilate. However, in cases of excessive oxidative stress, as occurs in disease states, reactive oxygen species or nitrosylation can change the oxidation state of sGC from normal reduced heme iron (Fe^{2+}) to oxidized heme (Fe^{3+}), making it less active and less responsive to NO. The oxidized sGC then loses its heme portion, after which it will eventually be degraded by the proteasome. The heme-free form of sGC is the target of sGC activators (Dasgupta et al., 2015). In the pulmonary circulation, NO acts as an endogenous pulmonary vasodilator and that synthesized by the action of eNOS on L-arginine in pulmonary vascular endothelial cells and then diffuses to adjacent vascular smooth muscle cells (VSMCs) to activate sGC (Triposkiadis et al., 2022). In VSMCs, MLCP, RhoA, RGS-2, IRAG, and BKCa are phosphorylated by PKG to promote vasodilation (Klinger and Kadowitz, 2017; Sadek et al., 2020). Many proteins are changed in their phosphorylation state when endogenous PKG is activated, including VASP and IRAG, Rap1B and Rap1Gap2, GRP2, and IP3 receptors, among others, which inhibit platelets as well (Gambaryan, 2022). Several studies have suggested vasoconstriction/vasodilation and proliferation/anti-proliferation alterations as possible pathogenic mechanisms of PAH (Biswas et al., 2020). PKG acts in pulmonary artery smooth muscle cells (PASMCs) by several mechanisms. PKG phosphorylates the BKCa channel and MLCK, leading to the relaxation of PASMCs (Christou and Khalil, 2022; Barenco-Marins et al., 2023). Vasodilator-stimulated phosphoprotein (VASP) is an actin-binding protein, and phosphorylation of VASP by PKG inhibits PASMC proliferation (Chen et al., 2004). Bone morphogenetic protein (BMP) is a signaling molecule belonging to the transforming growth factor-β (TGF-β) superfamily, which plays an important role in regulating cell proliferation, differentiation, and apoptosis. Recent studies have found that PKGI enhances the phosphorylation of the downstream signal small mothers against decapentaplegic (SMAD) 1/5 by BMP, promotes the antiproliferative and pro-differentiation effects of BMP, and keeps PASMCs in a proliferation-inhibited state (Watanabe, 2018).

The NO-sGC-cGMP signaling pathway maintains the normal function of the cardiovascular system in healthy individuals, and sGC activity falls as heart failure with reduced ejection fraction (HFrEF) progresses due to endothelial dysfunction and oxidative stress. sGC stimulation leads to increased cGMP synthesis, which can inhibit myocardial fibrosis, reduce vascular wall hardness, and induce vasodilation (Belenkov and Kozhevnikova, 2023). A lack of sGC stimulation and a decrease in cGMP production are associated with heart failure (HF) and decreased NO bioavailability. Elevated levels of plasma inflammatory cytokines, including TNF-α and IL-6, in patients with HF are associated with endothelial dysfunction with low NO-sGC-cGMP signaling in the heart and blood vessels (Numata and Takimoto, 2022). Impairment of the NO-sGC-cGMP pathway in HF, a key second messenger pathway mediating vascular and cardiac dilation, in patients with dysfunction of the endothelium, myocardium, and blood vessels may play a role in the progression of cardiovascular disease (CVD). Notably, both HFrEF and heart failure with preserved ejection fraction (HFpEF) patients have cGMP deficiency. There are also cases where oxidative stress results in heart muscle cell loss, collagen replacement, and fibrosis due to autophagy, apoptosis, or necrosis (Hulot et al., 2021). Disruption of the NO-sGC-cGMP pathway results in the narrowing of blood vessels, clumping of platelets, inflammation, scarring, and notably, maladaptive enlargement of the heart. Hence, NO-sGC-cGMP pathway restoration is a promising pharmacological target for HF treatment (Rudebusch et al., 2022). In the heart, PKG phosphorylates phospholamban (PLB) (Eggermont et al., 1988) and RyR2 (Xiao et al., 2006), thus activating sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) to promote the transport of calcium ions to the endoplasmic reticulum. In addition, PKG can phosphorylate numerous membrane channels, including L-type calcium channels (Yang et al., 2007) and transient receptor potential typical channel type 6. This ultimately reduces the inflow of extracellular calcium. Troponin I (Tsai and Kass, 2009), titin (Kruger et al., 2009), and CMyBP-C (Thoonen et al., 2015) act as structural proteins that regulate contraction of the myocardium, leading to myocardial relaxation through phosphorylation of PKG. PDE5 breaks down cGMP in cardiomyocytes into GTP, which is eventually recycled (Mihalek et al., 2022). Its activity counteracts vascular constriction and helps maintain vital organs, the highest expression of sGC being found in perfusion cardiomyocytes. cGMP produced by sGC causes ventricular diastole and decreased contractility (Hulot et al., 2021). In anti-myocardial hypertrophy, regulators of G-protein signaling (RGS), namely, RGS2 and RGS4, have a central role, leading to cGMP-mediated anti-myocardial hypertrophic effects by inactivating G-protein-coupled signaling, as RGS2 and RGS4 are targets of PKG (Klaiber et al., 2010). The Ca^{2+}-dependent mechanism of calcitonin/nFAT hypertrophy is further inhibited by the phosphorylation of the LTCC and transient receptor potential canonical channel type 6 (TRPC6) channels by cGMP/PKG (Kinoshita et al., 2010), and these mechanisms may be related to cGMP-mediated antihypertrophy, antifibrosis and alleviation of cardiac dysfunction (Sandner and Stasch, 2017).

Since the NO-cGMP signaling cascade is active in many tissues *in vivo*, the pathway is currently being explored for other roles in conditions other than cardiovascular disease, such as chronic kidney disease, fibrotic disease, neuroprotection, and dementia (Sandner, 2018). TGF-β signaling plays an important role in cellular fibrosis, and several preclinical studies have demonstrated that inhibition of TGF-β signaling exerts a potent antifibrotic effect in different organs in a variety of animal models (Distler et al., 2019; Englert et al., 2023). PKG inhibits the phosphorylation of extracellular signal-regulated kinase (ERK) by TGF-β signaling, suppresses ERK

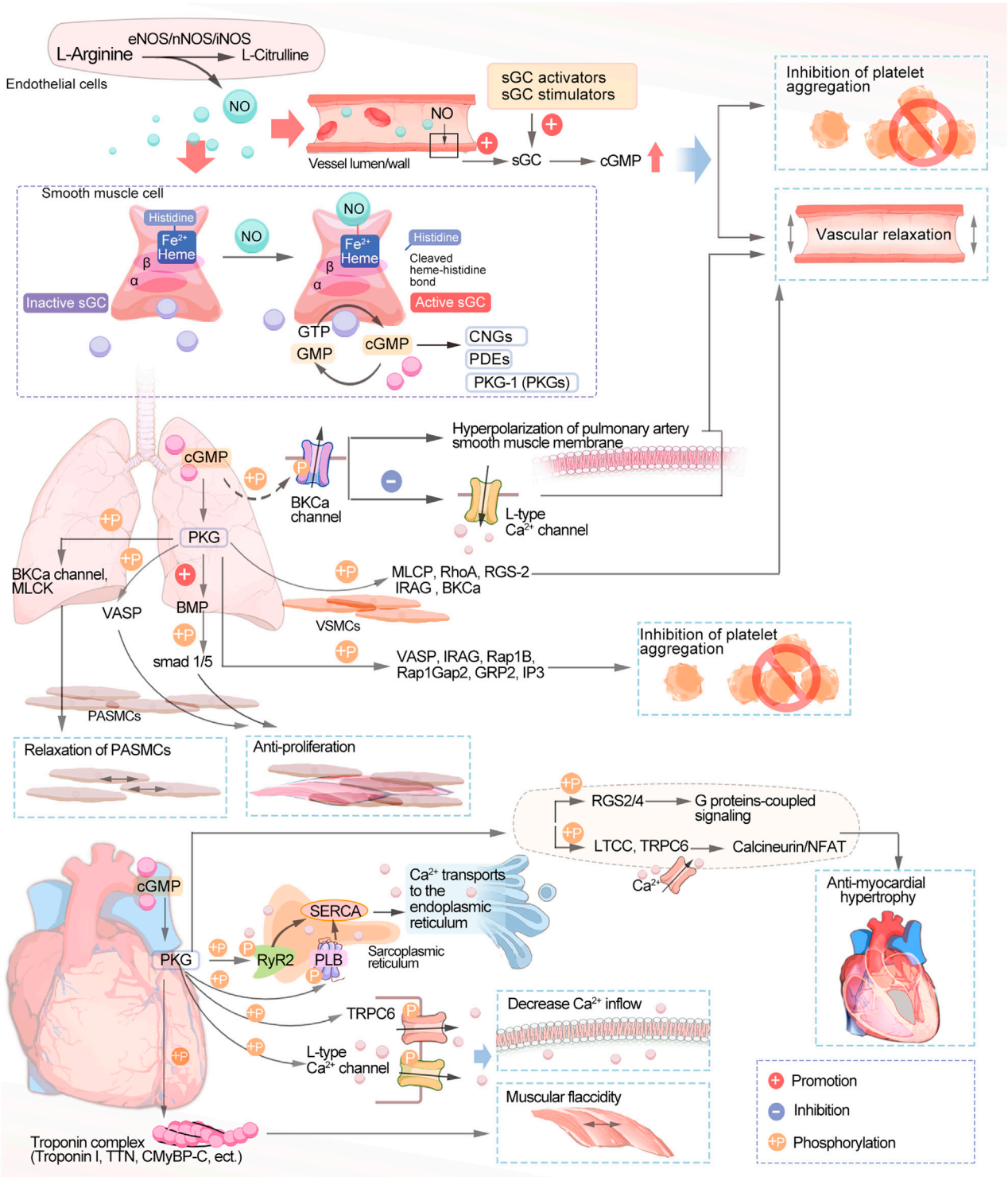

FIGURE 1
Schematic diagram of the NO-sGC-cGMP signaling pathway. NO, nitric oxide; sGC, soluble guanylate cyclase; cGMP, cyclic guanosine monophosphate; GTP, guanosine triphosphate; CNGs, cyclic nucleotide-gated ion channels; PDEs, phosphodiesterases; PKGs, protein kinases; BKCa, calcium-sensitive potassium channels; MLCK, myosin light chain kinase; VASP, vasodilator-stimulated phosphoprotein; BMP, bone morphogenetic protein; SMAD, small mothers against decapentaplegic; PASMCs, pulmonary artery smooth muscle cells; MLCP, myosin light chain phosphatase; RhoA, Ras homolog family member A; RGS-2, regulator of G-protein signaling 2; Rap1Gap2, Rap1 GTPase-activating protein 2; Rap1b, Ras-related protein 1; IRAG, IP3-induced calcium release; GRP2, guanyl-releasing protein 2; TTN, Titin; CMyBP-C, Cardiac myosin-binding protein-C; SERCA, sarco endoplasmic reticulum Ca^{2+}-ATPase; PLB, phospholamban; RyR2, cardiac ryanodine receptor; TRPC6, transient receptor potential canonical channel type 6; RGS, regulator of G protein signaling; LTCCs, L-type calcium channels; NFAT, calcineurin-nuclear factor of activated T cells.

signaling, and prevents its translocation to the nucleus, blocking TGF-β-mediated extracellular matrix (ECM) generation, fibroblast differentiation to myofibroblasts, and cell proliferation (Hu et al., 2017; Sandner and Stasch, 2017).

Furthermore, cGMP modulates renal blood flow, renin secretion, glomerular function, and tubular exchange processes through its direct effects on cGMP signaling cascades. It is possible to develop renal diseases such as chronic kidney disease (CKD) when NO-sGC-cGMP signaling is downregulated. As a result, therapeutic strategies that maintain or increase cGMP activity may be effective against progressive kidney disease (Krishnan et al., 2018).

Riociguat

Currently, riociguat is the only FDA-approved sGC stimulator for treating PAH and CTEPH. It is an orally administered drug that can be rapidly absorbed, with a bioavailability of 94.3%. Riociguat's half-life varies significantly from individual to individual, at approximately 12 h for PAH/CTEPH patients and 7 h for healthy people. Riociguat promotes cGMP synthesis. It has shown good efficacy in clinical trials and is well tolerated. Riociguat has a valuable place in the treatment of pulmonary hypertension (Kenny et al., 2022).

Riociguat in PAH

The IPATENT-1 trial (NCT00810693) found that riociguat significantly improved the 6-min walking distance of PAH patients by 36 m (m) over placebo. The PHIRST-1 trial (NCT00125918) and the SUPER-1 trial (NCT00644605) trials showed that riociguat had similar results to those of tadalafil (mean difference of 33 m) and sildenafil (mean difference of 50 m), which are both rivals of this product. Pharmacokinetics (PK) and adverse events (AEs) were also similar. Riociguat was used in the RESPITE study (NCT02007629) to treat PAH patients who failed to respond adequately to either tadalafil or sildenafil. Based on these findings, riociguat may outperform PDE-5 inhibitors in efficacy (Toxvig et al., 2019). Riociguat has not shown any new safety signals since the FDA approved it in 2013, according to an evidence-based review of its safety and tolerability. The overall incidence of AEs was 93%–96%. The most common AE types are nasopharyngitis and peripheral edema. The most common severe AEs (SAEs) include syncope, right ventricular (RV) failure, hypotension, and hemoptysis. Although common side effects are reported, they are tolerated in 87%–92% of people (Donaldson et al., 2020). It is also well tolerated in older patients (Barnikel et al., 2022). Study SE-IIP (NCT02138825) of idiopathic interstitial pneumonia with pulmonary hypertension (PH) was terminated early, as patients taking riociguat experienced a higher occurrence of SAEs and mortality, as well as no efficacy signal. In the main study, 11 patients died (8 in the riociguat group and 3 in the placebo group); riociguat was associated with more SAEs among PH-IIP patients, as well as adverse risk/benefit profiles. Therefore, patients with PH-IIP should avoid using riociguat (Nathan et al., 2019). This meta-analysis encompassed the incorporation of eight randomized controlled trials with 1,606 participants of riociguat's effects on all types of PH. For PAH and CTEPH patients, riociguat treatment significantly extended the 6MWD compared with placebo and decreased N-terminal pro-B-type natriuretic peptide (NT-proBNP), mean pulmonary artery pressure (PAP), pulmonary vascular resistance (PVR), and right atrial pressure (RAP). The cardiac index (CI) increased, cardiac output increased, and adverse events and clinical exacerbations decreased. A significant difference in efficacy outcomes and safety outcomes was not observed in other types of PH. Patients with PAH and CTEPH benefit from riociguat treatment, but patients with other types of PH only see partial hemodynamic improvements (Wang et al., 2021). The haemoDYNAMIC trial (NCT02744339) was a placebo-controlled, randomized, double-blind clinical trial that enrolled 114 patients with PH combined with HFpEF, who were randomly assigned to take riociguat or placebo. At 26 weeks, the riociguat group was significantly better in the primary efficacy measure, resting cardiac output, as determined through a right cardiac catheter (Dachs et al., 2022). In a multicenter, phase III, open-label, randomized controlled trial (NCT02634203), at week 26, riociguat therapy resulted in a more significant decrease in pulmonary vascular resistance than balloon pulmonary angioplasty (BPA) in 53 inoperable CTEPH patients, while BPA was performed on 52 patients. Treatment-related SAEs occurred more frequently in the BPA group: 22 of 52 (42%) of them experienced treatment-related SAEs vs. 5 of 53 (9%) in the riociguat group. Out of the 52 patients in the BPA group, 18 (35%) experienced lung injury, while 2 out of 53 patients (4%) reported severe hypotension leading to syncope. Deaths related to treatment were not reported (Jais et al., 2022). For patients requiring combination therapy, one review mentioned the possibility of pretreatment with riociguat plus an endothelin receptor antagonist in patients with PAH at high risk of death after 1 year (Rahaghi et al., 2023). A trial was conducted to test the combination of riociguat and ambrisentan in patients suffering from functional grade III PAH using a prospective, single-arm, open-label approach (NCT02634203) (Weatherald et al., 2022). In patients with sarcoidosis-associated pulmonary hypertension (SAPH), a double-blind placebo-controlled trial compared riociguat with placebo and examined the outcome of prolonged clinical worsening events (NCT02625558). Sixteen patients were randomized to riociguat (n = 8) and placebo (n = 8). By log-rank analysis, patients treated with riociguat stayed in the study significantly longer, their 6MWD scores tending to increase. At 1 year, riociguat was effective in preventing deterioration and in improving motor capacity in patients with clinical SAPH (Baughman et al., 2022). Riociguat is currently approved for use in adults only. It was tested for use in children in a multicenter, single-trial, 24-week, open-label phase 3 study, PATENT-CHILD (NCT02562235). The PK and safety of oral riociguat in pediatric patients with PAH were evaluated in World Health Organization Functional Classification (WHO-FC) Grade I-III patients aged 6–17 years who were being treated with stable endothelin receptor antagonists and/or prostacyclin analogs. They were given 0.5–2.5 mg of riociguat three times a day. A total of twenty-four individuals, with an average age of 12.8 years, were enrolled. Eighteen of these were WHO-FC II. Twenty patients (83%) reported primarily mild or moderate AEs. SAEs occurred in 4 cases (17%). All problems were resolved by the end of the study, and two out of the total (8%) were believed to be associated with the experimental medication. There were 3 cases of hypotension and 1 case of hemoptysis (all mild/moderate intensity). These children had similar blood concentrations of riociguat to those published in

adult patients. From baseline to week 24, the mean ± standard deviation of the 6-min walking distance of patients increased by 23 ± 69 m (n = 19), and the mean NT-proBNP decreased by −66 ± 585 pg/mL (n = 14). The WHO-FC of the patients did not change. A clinical worsening event occurred in two patients after they were hospitalized with right heart failure. Riociguat is a safe and effective treatment for pediatric PAH, based on the PK results. The data suggest that riociguat has an acceptable safety profile and a potential efficacy signal in a pediatric cohort (Garcia et al., 2022).

Anti-cardiac-remodeling effects of riociguat

To test the hypothesis that sGC stimulation with riociguat can prevent pathological cardiac remodeling and HF caused by chronic pressure overload, an animal model of C57BL/6N mouse HF was established. After 3 weeks of transverse aortic constriction (TAC) treatment, the animals were randomized to receive riociguat or its solvent (Sol). After 5 weeks of treatment, the left ventricular rejection fraction (LVEF) improved (TAC + Rio 43.4% ± 6.0%, TAC + Sol 20.9% ± 4.3%; $p < 0.001$), and the left ventricular mass to body weight (LVM/BW) ratio, myocardial fibrosis, and the myocyte cross-sectional area decreased (5.5 ± 0.4 mg/g vs 7.7 ± 0.7 mg/g; $p < 0.001$). RNA sequencing indicated that riociguat administration reduced the expression of myocardial remodeling genes (e.g., Nppa, Nppb, Myh7, collagen genes) and downregulated signaling pathways associated with hypertrophic cardiomyopathy and heart failure. Muscle and fibroblast cultures showed a reversal of pathological stress responses when riociguat was administered (Rudebusch et al., 2022). Another bioinformatic analysis of C57BL/6N mice subjected to TAC showed that riociguat administration improved the expression of markers of pathways, metabolism, and energy production related to cardiovascular disease induced by TAC. Changes in the levels of myosin heavy chain 7 (MYH7), cardiac phosphoprotein (PLN), and ankyrin repeat domain-containing protein 1 (ANKRD1) were reversed. Riociguat also attenuates TAC-induced changes in left ventricular microRNA levels. This suggests that riociguat has beneficial effects on cardiac structure and function during stress overload, supporting the potential use of riociguat as a novel treatment for HF (Benkner et al., 2022).

Riociguat in diffuse cutaneous systemic sclerosis (dcSSc) and peripheral arterial disease

The effectiveness and safety of riociguat in patients at high risk of progressing skin fibrosis in early dcSSc were evaluated in a randomized, double-blind, placebo-controlled phase 1b trial (NCT02283762), an 18-month study of 60 participants with a modified Rodman skin score (mRSS) of 10–22 units who received riociguat orally three times a day for 18 months (n = 60). At week 52, the change in mRSS units from baseline was −2.09 ± 5.66 (n = 57) in the riociguat group and −0.77 ± 8.24 (n = 52) in the placebo group (mean least squares difference was −2.34 (95% CI: −4.99–0.30) (p = 0.08). Among patients with interstitial lung disease, forced lung capacity decreased by 2.7% in the riociguat group and 7.6% in the placebo group. In the riociguat group, 41.3% (19 out of 46) of patients showed an improvement of 250% in their Raynaud scores at week 14, compared to 26.0% (13 out of 50) in the placebo group. In the safety assessment, no new signals of inflammation or treatment-related death were identified. Riociguat had no significant benefit on mRSS units. A secondary analysis and exploratory analysis revealed potential efficacy signals worth testing in further trials. Riociguat was well tolerated (Khanna et al., 2020).

Researchers have studied new blood vessel formation in mice with limb ischemia following the administration of riociguat. A 28-day treatment with 3 mg/kg/d riociguat was given intragastrically to C57BL/6 mice. Induction of posterior limb ischemia was achieved by surgically removing the femoral artery after 2 weeks of treatment. *In vitro* matrix tests showed that riociguat stimulated tubule formation in human umbilical vein endothelial cells (HUVECs) in a dose-dependent manner. Cell migration (assessed by scratch test) indices were also increased in HUVECs treated with riociguat compared to controls. At the cellular level, administration of riociguat resulted in swift initiation of the p44/p42 mitogen-activated protein kinase (MAPK) cascade in HUVECs. HUVECs treated with riociguat exhibited a reduced response to PKG inhibition. During ischemia, riociguat therapy improves blood flow recovery (as measured by laser Doppler imaging) and increases capillary density in ischemic muscle (as measured by CD31 immunostaining). The clinical results have shown that these indices reduce movement disorders and ischemic injuries significantly. The number of bone marrow-derived proangiogenic cells (PACs) was also increased by 94% when mice were treated with riociguat. Riociguat treatment was associated with significant improvements in PAC function, including migration ability, adherence to endothelial monolayers, and integration into the endodermal tubular network. GC stimulants can promote angiogenesis and improve neovascularization after ischemia, whose mechanism involves PKG-dependent p44/p42 MAPK activation, an improvement in PAC function, and an increase in their quantity. In patients with severe atherosclerosis, sGC stimulation may offer a novel approach to reducing tissue ischemia (Dhahri et al., 2023).

Vericiguat

Vericiguat was the first sGC stimulator to be marketed as of 2021, for patients with symptomatic chronic heart failure accompanied by an ejection fraction of 45%, to reduce the risk of cardiovascular death and heart failure hospitalization after heart failure hospitalization or the need for outpatient IV diuretics (Markham and Duggan, 2021). Vericiguat works synergistically with endogenous NO and enhances the affinity of sGC for low levels of NO (Singh et al., 2017). Therefore, vericiguat therapy is expected to restore the activity of an impaired NO-sGC-cGMP pathway, resulting in a variety of pharmacological effects, including improved cardiac and vascular function and reduced levels of profibrotic and inflammatory pathway markers. By increasing cGMP levels, vericiguat can also cause vascular relaxation and enhance the control of vascular tone and myocardial dysfunction (Hulot et al., 2021).

The average steady-state vericiguat distribution volume in healthy subjects is approximately 44 L, with a high bioavailability of approximately 93% when taken with food at a dose of 10 mg. Its serum albumin binding rate is approximately 98%, and its clearance rate is 1.6%. L/h. It takes 30 h for vericiguat to reach half-life in patients with HF. Ninety-five percent of the vericiguat dose is metabolized primarily by glucosylation of UGT1A1 (minor) and UGT1A9 (major), resulting in inactive N-glucuronic acid

metabolites. CYP450-mediated metabolism is a minor clearance pathway, accounting for only 5% of the total clearance capacity. Fifty-three percent of the metabolized drug is excreted in urine, and 45% is excreted in feces (Campbell et al., 2022; Chiles and Al-Horani, 2022). Vericiguat, at doses up to 10.0 mg QD for 7 days, is generally well tolerated in healthy men in Europe, China, and Japan. Oral vericiguat 15.0 mg was not well tolerated, and drug-related treatment-emergent adverse events (TEAEs) were predominantly neurologic disorders such as headache and postural dizziness, which may be related to the mode of action of vericiguat (namely, vasodilation) (Boettcher et al., 2021).

Vericiguat in HFrEF

Preliminary evidence regarding vericiguat in the context of HFrEF was derived from two clinical trials, namely, the phase II study SOCRATES-REDUCED and the phase III study VICTORIA (Vannuccini et al., 2022). Four hundred fifty-six patients with clinical stability with an LVEF less than 45% within 4 weeks of worsening chronic heart failure, defined as congestion symptoms and elevated natriuretic peptide levels requiring inpatient or outpatient intravenous diuretics, were randomly assigned to an arm of the SOCRATES-REDUCED trial (NCT01951625). Overall, 351 patients (77.0%) completed investigational drug therapy with 12 weeks of effective NT-proBNP levels without major protocol bias, meeting the criteria for primary endpoint evaluation. In the preliminary analysis, the AE rates were 77.2% and 71.4% in the placebo and 10 mg vericiguat groups, respectively. The effects of vericiguat on NT-proBNP levels at 12 weeks in patients with chronic heart failure exacerbations and reduced LVEF were not significant compared with placebo, but the vericiguat was well tolerated. This drug had a dose–response relationship, indicating that further clinical trials will be needed to determine the effectiveness of the drug in treating worsening chronic heart failure patients (Gheorghiade et al., 2015). In the VICTORIA III trial (NCT02861534), 5,050 patients were diagnosed with chronic heart failure (class II, III, or IV according to the New York Heart Association) with an ejection fraction of less than 45%. They were given vericiguat (10 mg once daily, target dose) or placebo (no treatment), along with guideline-based medication. The combined result of cardiovascular death and the first hospitalization for heart failure was the primary outcome. At a median of 10.8 months, 897 of 2,526 patients (35.5%) in the vericiguat group had had a primary outcome event, compared with 972 of 2,524 patients (38.5%) in the placebo group (hazard ratio, 0.90; 95% CI, 0.82–0.98; p = 0.02). A total of 691 (27.4%) patients were hospitalized for heart failure in the vericiguat group *versus* 747 (29.6%) patients in the placebo group (hazard ratio 0.90; 95% CI, 0.81–1.00). There were 414 (16.4%) deaths from cardiovascular causes in the vericiguat group and 441 (17.5%) deaths from cardiovascular causes in the placebo group (hazard ratio, 0.93; 95% CI, 0.81–1.06). A total of 957 (37.9%) patients died from any cause or were hospitalized for heart failure in the vericiguat group *versus* 1,032 (40.9%) patients in the placebo group (hazard ratio: 0.90; 95% CI, 0.83–0.98; p = 0.02). Symptomatic hypotension occurred in 9.1% and 7.9% of patients in the vericiguat and placebo groups (p = 0.12), respectively, and syncope occurred in 4.0% and 3.5% of patients in the vericiguat and placebo groups (p = 0.30), respectively. In conclusion, among high-risk patients with heart failure, treatment with vericiguat led to a lower incidence of death from cardiovascular causes or hospitalization for heart failure than placebo (Armstrong et al., 2020a).

Vericiguat in HFpEF

Socrates-preserved (NCT01951638) was a 12-week, double-blind, placebo-controlled, phase 2b trial of 447 patients with deteriorating symptoms of chronic hypertension and an LVEF ≥45% (Pieske et al., 2017). Patients were randomized to receive placebo or vericiguat once daily (1.25, 2.5, 2.5–5.0, or 2.5–10.0 mg). Patients tolerated vericiguat well (AEs: vericiguat 10 mg arm, 69.8%; placebo, 73.1%), but the two groups had similar changes from baseline in NT-proBNP (one-sided p = 0.90, two-sided p = 0.20) and left atrial volume (one-sided p = 0.81, two-sided p = 0.37) at 12 weeks. A larger proportion of patients treated with 10 mg vericiguat achieved clinically meaningful improvements in the Kansas City Cardiomyopathy Questionnaire Clinical Summary Score (KCCQ-CSS) and the 5-dimensional EuroQol questionnaire (EQ-5D). Given the encouraging results in terms of quality of life, there is a need for further studies on the impact of vericiguat in patients with HF. The VITALITY study (NCT03547583) was designed to understand the efficacy and safety of vericiguat on quality of life and exercise tolerance in patients with HF and HFpEF (Armstrong et al., 2020b). A total of 789 patients had chronic HF, EF ≥ 45%, New York Heart Association functional class II or III, decompensation in the last 6 months (no hospitalization due to HF or need for diuretics for intravenous treatment of HF), and elevated natriuretic peptides. Patients were randomized to receive up to 15 mg (n = 264), 10 mg (n = 263) or placebo (n = 262). No significant differences were found between groups in the physical limitation score of the KCCQ or the 6MWD after 24 weeks of treatment. The different outcomes between SOCRATES-PRESERVED and VITALITY in terms of improving quality of life in patients with HFpEF are of interest and require further investigation.

Vericiguat in chronic coronary syndromes

Vericiguat plus nitroglycerin was compared to nitroglycerin alone for safety, tolerability, and pharmacodynamic effects in patients with chronic coronary syndromes (CCSs). The VENICE (NCT02617550) randomized, double-blinded, phase I, multicenter trial randomized 36 patients with CCSs to receive either 2.5 mg vericiguat (increased doses every 2 weeks to 5 mg and 10 mg) or placebo. There were 31 patients in the study (21 receiving vericiguat plus nitroglycerin; 10 receiving placebo plus nitroglycerin). The combination of vericiguat with nitroglycerin did not increase the number of adverse events nor the risk of SAEs in patients with CCSs (Boettcher et al., 2022).

Praliciguat

Praliciguat (also known as IW-1973) is an sGC stimulator in the clinical stage of testing. It is being studied in clinical studies for the treatment of heart failure and diabetic nephropathy with retained ejection. Treatment with 1–10 mg/kg IW-1973 significantly reduced blood pressure in rats with normal and spontaneous hypertension in nonclinical models. IW-1973 reduced blood pressure, inflammatory

factors, and markers of kidney disease, such as proteinuria and renal fibrosis, in rat models. IW-1973 has a wide tissue distribution. It shows renoprotective, anti-inflammatory, and antifibrotic effects (Tobin et al., 2018). In a randomized, placebo-controlled phase I study, different doses of praliciguat were evaluated for safety, tolerability, PK, and pharmacodynamics (PD) in healthy adults (n = 44). It was tolerable at various doses, and no SAEs were reported. The most common adverse reactions were headaches and decreased blood pressure. The PK was proportional to the dose, and the effective half-life was 24–37 h. The administration of praliciguat resulted in a rise in plasma cGMP that was proportional to the dosage, indicating activation of sGC. Repeated daily medication can lead to a drop in blood pressure (Hanrahan et al., 2019).

Praliciguat in HFpEF

There are no approved sGC stimulants for treating HFpEF, but such treatments are being investigated. CAPACITY HFpEF (NCT03254485) is a phase 2, multicenter, randomized, double-blind, placebo-controlled, parallel-group trial designed to evaluate the safety and efficacy of approximately 181 patients with HFpEF over 12 weeks. A total of 155 participants have completed the trial. The praliciguat group (n = 65) showed a change in the peak rate of oxygen consumption (Vo2) of −0.26 mL/kg/min (95% CI, −0.83 to 0.31), compared to 0.04 mL/kg/min (95% CI, −0.49–0.56) in the placebo group (n = 78). Changes in the 6MWD were 41.4 m (95% CI, 8.2–74.5) and 58.1 m (95% CI, 26.1–90.1), respectively. No significant benefit of praliciguat was observed in HFpEF compared to placebo over a 12-week follow-up period, but it was accompanied by more hypotension and headache. These findings do not support the use of praliciguat in patients with HFpEF (Udelson et al., 2020).

Praliciguat's different effects in patients with T2D

Praliciguat has renoprotective properties. In a rat model of obese diabetic nephropathy (DN), praliciguat alone reduced proteinuria. Praliciguat monotherapy had no effect on hemodynamics, whereas combined enalapril reduced proteinuria, but monotherapy reduced blood pressure and did not reduce proteinuria (Liu et al., 2020). In a phase II trial (NCT03217591) involving 156 adults with type 2 diabetes, praliciguat did not significantly reduce proteinuria over 12 weeks (Hanrahan et al., 2020b). Furthermore, a mouse model of diet-induced obesity revealed some beneficial metabolic effects of praliciguat (Schwartzkopf et al., 2022). Another phase II, double-blind, placebo-controlled trial of praliciguat (NCT03091920) involved 26 patients with type 2 diabetes combined with hypertension. Praliciguat was well tolerated and showed positive trends in metabolic and BP variables (Hanrahan et al., 2020b).

One study evaluated the effect of praliciguat on hind limb ischemic (HLI) recovery in mice with type 2 diabetes. Praliciguat significantly increased the diameter of their small arteries, decreased the expression of intercellular adhesion molecule 1 (ICAM1), prevented the accumulation of oxidative proangiogenic and proinflammatory muscle fibers, and significantly downregulated the expression of Myh2 and Cxcl12 mRNA in cultured myoblasts (Foussard et al., 2023).

Other medicines

BAY-747

BAY-747 is a long-acting, next-generation GC stimulator for the treatment of refractory hypertension that is administered once daily and has sustained effects on blood pressure and heart rate (up to 24 h). BAY-747 is taken orally in a single dose of 0.5–15 mg in the form of a polyethylene glycol (PEG) solution and is well tolerated in healthy volunteers. Blood concentrations of BAY-747 peaked within 2–6 h, independent of dose intensity. A single dose of 3.5 mg oral BAY-747 significantly increased heart rate and reduced blood pressure and mean arterial pressure, these effects being most pronounced within the first 4 h after taking the study drug. A single oral dose of 10 mg BAY-747 had significant effects on heart rate, cardiac output, and cardiac index, with maximum effects achieved within 4 h of administration. In the 0.5–20 mg dose range, a single oral dose of BAY-747 did not appear to affect stroke volume (Vakalopoulos et al., 2023).

Olinciguat

Olinciguat is a new oral sGC stimulator currently in phase II clinical development (NCT02931565 and NCT03285178). Olinciguat has cardioprotective effects and reduces blood pressure. In addition, it has renal-protective effects, and in a rat ZSF1 model, there is a correlation between decreased levels of glucose, cholesterol, and triglycerides (Zimmer et al., 2020). In a mouse model of TNFα-induced inflammation, olinciguat treatment was associated with reduced levels of soluble adhesion derived from endothelial cells and white blood cells (Tchernychev et al., 2021). Accordingly, it may be suitable for treating diseases characterized by vascular and extravascular lesions as well as a wide range of potential therapeutic applications.

MK-2947

MK-2947 is a novel, potent, selective sGC stimulator (Brockunier et al., 2020). A pharmacological study demonstrated that MK-2947 effectively ameliorates angiogenic performance and blunts the myofibroblast-like profibrotic phenotype of SSc dermal microvascular endothelial cells (SSc-MVECs), thus providing new evidence for the benefit of repurposing sGC stimulators for SSc (Romano et al., 2023).

CYR715

CYR715, first described by Rennie et al., is a novel carboxylic acid-containing sGC stimulator that exhibited similar dose-dependent hemopharmacology in normotensive rats. Compared to the previously described IWP-051, CYR715 had a better pharmacokinetic profile in rats and exhibits similar dose-dependent hemodynamics in normotensive rats (Rennie et al., 2021). A recent study found that preincubating red blood cells from type 2 diabetes (T2D) patients with CYR715 and administering them to isolated rat hearts enhanced left ventricular diastolic pressure recovery, reduced infarct size, and mitigated endothelial dysfunction. Therefore, CYR715 appears to be an attractive therapeutic strategy for preventing cardiovascular injury in patients with T2D (Jiao et al., 2023).

6MWD, 6-min walking distance; PAH, pulmonary arterial hypertension; CTEPH, chronic thromboembolic pulmonary hypertension; PVR, pulmonary Vascular resistance; IIP, idiopathic interstitial pneumonias; dcSSc, diffuse cutaneous systemic sclerosis; AEs, adverse events; SAEs, serious adverse events; mRSS, modified Rodnan skin score; HF, heart failure; NT-proBNP, N-terminal pro-B-type natriuretic peptide; HF, heart failure; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LAV, left atrial volume; KCCQ, Kansas City Cardiomyopathy Questionnaire; CCSs, chronic coronary syndromes; TEAEs, treatment-emergent adverse events; HFpEF, heart failure with preserved ejection fraction; ABPM, ambulatory BP monitoring; HOMA-IR, homeostatic model assessment of insulin resistance; SAPH, sarcoidosis associated pulmonary hypertension; ADEs, adverse events resulting in study drug discontinuation; BFT, supine bolus flow time.

Soluble guanylate cyclase activators

While sGC stimulators target reduced and heme-containing forms of sGC, sGC activators target oxidized or heme-free sGC. Since the status of these enzymes occurs mainly in the condition of diseases accompanied by oxidative stress, this binding pattern is quite attractive for the clinical use of activator drugs (Evgenov et al., 2006). Cinaciguat, a type of amino dicarboxylic acid, is the first characteristic drug of this new sGC activator class (Stasch et al., 2002). Although other sGC activators have been identified, no sGC activators are available to patients.

Cinaciguat

A 1997 ultrahigh-throughput screening (uHTS) identified cinaciguat as an sGC activator (Sandner et al., 2021b). As a direct, NO-independent activator of sGC, cGMP levels directly increase in the presence of heightened oxidative stress and impaired endothelial function, which could yield notable efficacy. However, it increases the risk of low blood pressure (Mitrovic et al., 2011).

Cinaciguat in PAH

In a randomized, double-blind, multicenter, multinational phase IIb study (NCT00559650), cinaciguat significantly reduced pulmonary capillary wedge pressure (PCWP) and mean right atrial pressure in patients with decompensated chronic congestive heart failure. There was also a decrease in both pulmonary and systemic vascular resistance, as well as a reduction in mean arterial pressure, and the cardiac index increased (Mitrovic et al., 2011). Cinaciguat was associated with 71% of adverse events, and placebo was associated with 45%. There were no adverse events associated with 30-day mortality. When the dose was increased to 200 g/h, hypotensive events increased, and the trial was terminated (Erdmann et al., 2013).

Cinaciguat in acute HF

Three phase IIb trials, including COMPOSE 1 (NCT01065077), COMPOSE 2 (NCT01067859), and the COMPOSE Early Trial (NCT01064037), were subsequently conducted to investigate the safety and efficacy of varying doses of cinaciguat 200 μg/h *versus* placebo in treating patients with acute HF initiated at different time points. However, because hypotensive events occurred and no significant benefit was observed, the clinical development of cinaciguat was discontinued (Breitenstein et al., 2017). The clinical trials led to the discontinuation of cinaciguat. Most of the research since then has been conducted on animals (Benaldo et al., 2022; Dai and Stuehr, 2023).

Other medicines

Ataciguat

Ataciguat, formerly known as HMR 1766, is an anthranilic acid derivative that is a novel sGC activator (Schafer et al., 2010). Researchers found that ataciguat normalized vasodilation and the vascular response to exogenous NO in rats with congestive heart failure and reduced platelet activation (Schafer et al., 2010). In a rat model of inflammatory chronic renal impairment, ataciguat exhibited beneficial BP-independent effects on kidney structure and urinary albumin excretion (Benz et al., 2007). Ataciguat is currently being studied in clinical trials for numerous indications, including changes in tolerated blood pressure and orthostatic tolerance (i.e., ability to stand without passing out) in patients with mild to moderate calcified aortic stenosis (NCT02049203), effectiveness in relieving patients with neuropathic pain (NCT00799656), improving claudication in patients with PAD (NCT00443287), and slowing the progression of valve calcification in patients with moderate calcific aortic valve stenosis (NCT02481258). However, the results of these clinical trials have not yet been made publicly available.

MGV354

MGV354 is a novel sGC activator that effectively lowers IOP in glaucoma models in preclinical studies (Prasanna et al., 2018). Unfortunately, in a clinical trial (NCT02743780), MGV354 did not cause a statistically significant reduction in IOP compared to placebo (Stacy et al., 2018).

Mosliciguat

Mosliciguat (BAY 1237592) is an sGC activator designed for topical application in the lung for the treatment of PAH. Inhalation of mosliciguat specifically activates apo-sGC, leading to a selective effect in the lung (Becker-Pelster et al., 2022). Mosliciguat was shown to activate heme-free NO-GC and improve cardiopulmonary circulation in minipigs and rats (Becker-Pelster et al., 2022). Based on these results, mosliciguat is currently in phase Ib clinical development (NCT03754660) as an inhaled therapy for PAH.

Runcaciguat

As a once-daily oral sGC activator, runcaciguat demonstrated good PK distribution when administered by Bayer (Hahn et al.,

2021). Preclinical studies have demonstrated that runcaciguat may be effective in preventing CKD caused by hypertension, diabetes, and obesity (Benardeau et al., 2021). The oral sGC activator runcaciguat is currently in a phase II clinical program for patients with proteinuric CKD (NCT04507061).

BI 685509

BI 685509 is an orally bioavailable, potent sGC activator that exhibits significant renal protection properties and antifibrotic activity in preclinical models of kidney disease and injury (Reinhart et al., 2023). In addition, in a preclinical rat model of thioacetamide-induced nodularity, hepatic fibrosis, portal hypertension and portosystemic shunts, BI 685509 reduced Sirius red morphometry (SRM) by 38%, alpha-smooth muscle actin (αSMA)-positive area by 55%, portal pressure by 26% and portal shunt by 10%; hence, it could be used as a potential treatment for cirrhosis-associated portal hypertension (Jones et al., 2023). BI 685509 effectively inhibited the induction effect of activated platelet-rich plasma on the SSC-related chemokine CXCL4, more strongly than riociguat did (Nabozny et al., 2023). These findings suggest that BI 685509 is a new drug to treat SSc that is superior to riociguat. In a phase Ib study (NCT03165227), BI 685509 was generally well tolerated in patients with diabetic kidney disease (DKD) (Cherney et al., 2023). BI 685509 is currently in phase II trials for CKD (NCT04736628) and DKD (NCT04750577). Studies are currently underway on the indications of PAH and CTEPH (NCT03754660) and SSc (NCT05559580).

BI 703704 and GSK2181236A

The sGC activators BI 703704 and GSK2181236A have demonstrated sustained protection against preclinical models of CKD (Costell et al., 2012; Stasch et al., 2015; Hu et al., 2022). The results of these preclinical studies need to be confirmed in humans before these agents can be considered alternatives to current recommended treatments.

Guanylate cyclase-C agonists

GC-C is a transmembrane protein receptor that has received increasing attention for its importance in digestive diseases. It plays a key role in regulating water and electrolyte balance, maintaining gastrointestinal function, relieving abdominal pain, controlling inflammation, regulating intestinal ecology, inhibiting tumor growth and regulating cell proliferation and is considered a potential therapeutic target for digestive system diseases (Waldman and Camilleri, 2018). It was discovered in 1970 as a receptor for heat-stable endotoxins of exogenous diarrhea-causing bacteria and can be detected not only in intestinal mucosal cells but also in primary and metastatic colorectal cancer, peripheral blood, lymph nodes and liver tissues (Smith and Gyles, 1970). The signaling pathway of GC-C/cGMP has a significant impact on digestive disorders, and agonists are on the market to treat these associated gastrointestinal conditions. GC-C agonists include natural and synthetic ligands; natural ligands include endogenous ligands such as uroguanylin and guanylin, exogenous ligands such as heat-resistant enterotoxin, and synthetic ligands such as linaclotide, plecanatide and dolcanatide (Kuhn, 2016). The current progress of GC-C agonists is summarized in Table 3.

The GC-C/cGMP signaling pathway

As shown in Figure 2, intestinal guanylin and uroguanylin are effective regulators of fluid ion homeostasis. They are secreted by various cells of the intestinal mucosa, including intestinal chromaffin cells, epithelial cells, goblet cells, and Pan's cells. These peptide hormones act as receptor GC-C ligands that produce intracellular cGMP and activate PKGII. PKGII phosphorylates the cystic fibrosis transmembrane conduction regulator (CFTR) and increases the secretion of chloride ions (Cl^-) into the intestinal lumen. cGMP can also increase the content of cyclic adenosine monophosphate (cAMP) by inhibiting the activity of PDE3. cAMP activates PKA and coactivates CFTR along with PKGII. Promoting the discharge of chloride ions and bicarbonate (Vaandrager, 2002), cGMP activates HCO_3^- secretion through an unknown mechanism. In addition, cGMP can inhibit the sodium/hydrogen exchanger NHE3, thereby reducing the absorption of sodium, preventing hypernatremia and unnecessary hypervolemic shock, and maintaining the fluid balance in the intestine. By these mechanisms, cGMP can maintain the hydration state of colon mucus and ion homeostasis (Brierley, 2012; Waldman and Camilleri, 2018; Samanta and Chaudhuri, 2021). In addition, it can regulate the intestinal immune barrier and increase the levels of IL-2 and IFN-γ and paracellular permeability (Xing et al., 2021). Abdominal pain is a major symptom of inflammatory bowel disease (IBD), and therapies modulating the GC-C/cGMP pathway promote visceral analgesia in patients with these diseases in animal models and clinical trials (Waldman and Camilleri, 2018). The mechanism of its analgesia is mainly through the afferent pathway involved in the regulation of gastrointestinal pain. After receptor–ligand binding in epithelial cells, elevated intracellular cGMP can be transported to the extracellular space through the silencing of the multidrug resistance-related protein 4 cyclic nucleoglycine efflux pump located on the basolateral membrane, reducing the excitation of submucosal afferent neurons and relieving abdominal pain. Another mechanism underlying this action is the prevention of intraluminal factors from affecting pain afferents and immune mechanisms in the lamina propria and other areas to indirectly promote analgesia (Shailubhai et al., 2015; Brierley et al., 2022). It can also inhibit the proliferation of intestinal epithelial cells and maintain genomic stability, thus inhibiting the occurrence of intestinal tumors. Following the activation of GC-C, the level of cGMP increases to activate cyclic phospho-dependent PKGII, which can inhibit the protein kinase B (Akt)-related signaling pathway and thus upregulate the expression of the tumor suppressor p53. p38 MAPK is also activated, which increases the phosphorylation of the transcription factor Sp1, leading to the accumulation of p21 in cells, which promotes cell aging and reduces the risk of cancer (Basu et al., 2014). Activation of GC-C induces apoptosis by promoting the degradation of β-catenin and opposing the pro-proliferative Wnt/β-catenin/Tcf-4 signaling pathway (Thompson et al., 2000). Because Akt enhances β-catenin activity either directly or indirectly, the suppression of Akt signaling may also be associated with the reduction of β-catenin/T-cell factor

TABLE 3 The current progress on guanylate cyclase-C agonists.

Drug	Trial name	Design	Population	Dose	Trial ID	Endpoint	Safety outcome	Stage of development	Conclusion	Reference
Linaclotide	/	Phase III, International, Multicenter, Randomized, Double-blind, Placebo-controlled, Parallel-group Trial	Patients with IBS-C	290 μg daily	NCT01880424	Composite Endpoint of Abdominal Pain and IBS	AEs	Completed	Linaclotide was efficacious and well-tolerated in Chinese Patients with IBS-C, with rapid onset of effect	Liang and Liang (2023)
Linaclotide	/	Phase IIIb, Randomized, Double-blind, Placebo-controlled, Parallel-group Trial	Patients with IBS-C	290 μg daily	NCT03573908	Change in abdominal Score	TEAEs	Completed	Linaclotide significantly reduced multiple abdominal symptoms important to patients with IBS-C	Brenner et al. (2023)
Linaclotide	/	Phase II Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel Group Trial	Patients with OIC	145 μg or 290 μg daily	NCT02270983	change in SBMs/week	AEs and SAEs	Completed	Linaclotide significantly improved OIC symptoms and was well tolerated in patients with chronic noncancer pain	Brenner et al. (2020)
Linaclotide	/	Phase I, Randomized, Placebo-Controlled Trial	Patients with Colorectal Cancer	0.87 mg daily	NCT01950403	difference in mean cGMP levels after 7 days	AEs	Completed	Linaclotide was associated with homeostatic signaling, including phosphorylation of vasodilator-stimulated phosphoprotein and inhibition of proliferation quantified by fewer Ki67-positive epithelial cells	Weinberg et al. (2017)
Linaclotide	/	Phase III Randomized, Double-blind, Placebo-controlled trial	Patients with CC	0.5 mg daily	NCT02809105	Change from baseline in average weekly SBM frequency	AEs 、 SAEs	Completed	Linaclotide 0.5 mg/day is effective and safe in Japanese CC patients	Fukudo et al. (2019)
Plecanatide	The CIC3 Study	Randomized, Double-Blind, Placebo-Controlled trial	Patients with CIC	3 mg or 6 mg daily	NCT01982240	percentage of CSBM	AEs	Completed	Plecanatide significantly improved constipation and related symptoms with a low rate of adverse events	Miner et al. (2017)
Plecanatide	The National CIC3 Study	Randomized, 12-Week, Double-Blind, Placebo-Controlled trial	Patients with CIC	3 mg or 6 mg daily	NCT02122471	Number of Durable Overall CSBM Responders	AEs 、 SAEs and TEAEs	Completed	Plecanatide has a positive efficacy and safety profile in CIC patients	DeMicco et al. (2017)

IBS-C, irritable bowel syndrome with constipation; AEs, adverse events; SAEs, serious adverse events; TEAEs, treatment-emergent adverse events; OIC, opioid-induced constipation; SBMs, spontaneous bowel movements; CIC, chronic idiopathic constipation; CSBM, complete spontaneous bowel movement; CC, chronic constipation.

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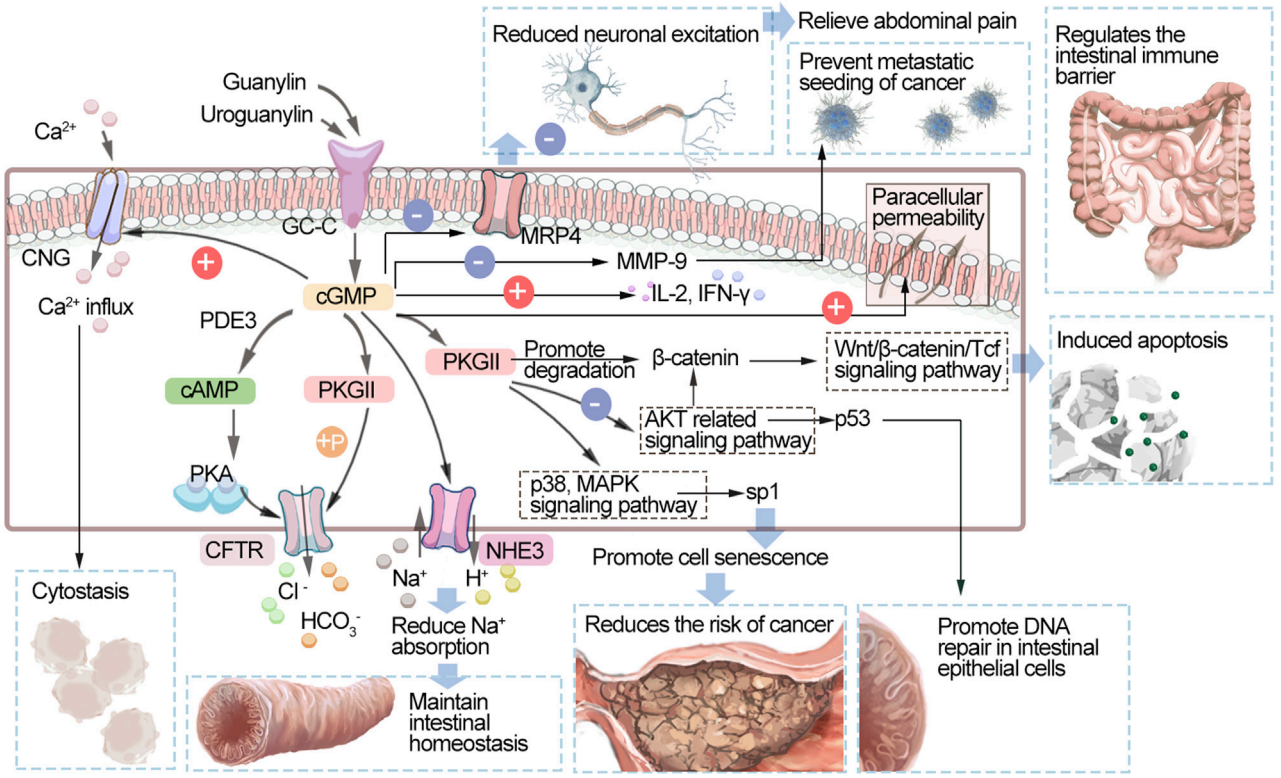

FIGURE 2
Schematic diagram of the GC-C/cGMP signaling pathway. GC-C, guanylate cyclase C; cGMP, cyclic guanosine monophosphate; PDEs, phosphodiesterases; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PKG, protein kinase; CNGs, cyclic nucleotide-gated ion channels; CFTR, cystic fibrosis transmembrane regulator; NHE3, Na^+/H^+ exchanger 3; MRP4, multidrug resistance-associated protein 4; Akt, protein kinase B; p38 MAPK, p38 mitogen-activated protein kinase; MMP-9, matrix metalloproteinase-9; IL-2, interleukin 2; IFN-γ, Interferon γ.

(TCF) transcriptional activity mediated by GC-C (Fang et al., 2007). As the GMP concentration increases, cyclic nucleotide-gated ion channels (CNGs) are activated, Ca^{2+} inflow is promoted, and cytostatic activity is mediated (Kazerounian et al., 2005). GC-C signaling can also block metastasis by inhibiting matrix metalloproteinase-9 (MMP-9), which is produced by colorectal cancer cells (Lubbe et al., 2009). PKGII can also promote DNA repair in intestinal epithelial cells, thereby protecting intestinal epithelial tight junction proteins, maintaining intestinal wall integrity and reducing the risk of tumor development (Rappaport and Waldman, 2020; Loretah et al., 2021).

Linaclotide

Linaclotide, a 14-amino acid peptide, was approved by the FDA in 2012 as an oral drug for the treatment of chronic constipation (CC) and constipation-type irritable bowel syndrome (IBS-C). It acts on the GC-C receptor on the luminal membrane, resulting in increased intracellular phosphorylation of cGMP and pVASpser239. The mechanisms underlying its effects include increased bicarbonate secretion during CFTR expression or functional loss, increased chloride and bicarbonate secretion into the intestine, inhibited sodium ion absorption, and thus increased water secretion into the lumen and improved defecation (Love et al., 2014; Ahsan et al., 2017; Sarthi et al., 2023). The pharmacological activity of linaclotide is limited to the gastrointestinal tract, and its oral bioavailability is low, so systemic side effects are unlikely. After oral linaclotide is taken, approximately 3%–5% of the active peptide is excreted in the stool. Linaclotide and its metabolites are excreted through the kidneys (33%–45%) and biliary tract (48%–59%). Its dosage should be adjusted with particular care in patients with moderate liver impairment or mild to severe liver impairment (data are not available for patients with severe liver impairment). It is metabolized by multiple cytochrome P450 (CYP) enzymes and has no effect on major CYP subtypes, giving it a low risk of clinically relevant drug interactions (Frey et al., 2018). A therapeutic dose of linaclotide should be taken at least 30 min before meals, as effectiveness and tolerability can be affected by high-fat foods (Bassotti et al., 2018).

Linaclotide in chronic constipation syndrome

Linaclotide was evaluated at doses between 62.5 g and 600 g for efficacy and safety in patients (n = 4,107) with CC in a meta-analysis. More patients completed spontaneous defecation (CSBM) at different doses, which was more significant in the very low-dose group, and there were no adverse reactions in the high-dose group (Yang and Lei, 2021). Japanese researchers (NCT02809105) found that linaclotide at a dose of 0.5 mg/day was effective and safe in patients with CC, with mild and occasional adverse reactions being the most common (Fukudo et al., 2019). A prospective study from China evaluated patients taking linaclotide (n = 97) on bowel movements, abdominal symptoms, IBS Symptom Severity Scale (IBS-SSS), and IBS Quality of Life Questionnaire (IBS-QOL) and found a significant increase in weekly

bowel movements and a significant improvement in patients' quality of life. Diarrhea occurred in 11 cases (11.3%). IBS-C symptoms and severity were improved with linaclotide, and the drug was safe and effective (Liu et al., 2022). Another phase III clinical trial involving 659 Chinese IBS-C patients also showed that linaclotide (290 μg/day) was effective and well tolerated in Chinese IBS-C patients with rapid onset of action (NCT01880424) (Peng et al., 2022). Another study found that linaclotide reduced reaction time in patients with IBS-C (Brenner et al., 2023). In a trial in the elderly population, the most common side effect was diarrhea, but the incidence of diarrhea and constipation in elderly patients, even at reduced doses, did not differ significantly from that in nonelderly patients, and multivariate analysis showed that age, sex, and dose were not associated with diarrhea caused by linaclotide treatment. Thus, linaclotide was effective and safe in elderly patients (Chang et al., 2021; Ishigo et al., 2021). In special pediatric populations, AEs are relatively common, although studies have found that nearly half of children with FC or IBS-C benefit from linaclotide treatment, so further research is needed (Baaleman et al., 2021). In a cohort study of patients with SSc, 31 patients were treated with linaclotide. Twenty-eight of the 31 patients responded to treatment, while only three (9.7%) reported ineffective or intolerable side effects. Diarrhea, cramping, and bloating were the most commonly reported side effects (11/31, 35%). Linaclotide is a well-tolerated, effective drug that can be used to treat refractory symptoms of a low GI score on SSc (Dein et al., 2021). A multicenter phase II clinical study evaluated the efficacy and safety of linaclotide in the treatment of opioid-induced constipation (OIC) in patients with chronic noncancer pain syndrome (NCT02270983). Compared with placebo, linaclotide significantly improved stool consistency, diarrhea, bloating, and treatment satisfaction scores ($p < 0.05$). Linaclotide significantly improved OIC symptoms and was well tolerated in patients with chronic noncancerous pain (Brenner et al., 2020).

Linaclotide in visceral pain and colon cancer

Linaclotide treatment was found to reduce vaginal hyperalgesia and mechanical hyperalgesia associated with endometriosis through viscerovaginal crosstalk (Ge et al., 2019). In patients with colon cancer, a US phase I clinical trial (NCT01950403) showed that administration of linaclotide (870 μg/d) for 7 days after oral preparation of the intestine with polyethylene glycol increased the level of cGMP and reduced the proportion of Ki-67-positive colon epithelial cells (a higher proportion of Ki-67-positive cells suggested a faster rate of cell proliferation). These results suggest that linaclotide inhibits colonic epithelial cell proliferation in human colons (Weinberg et al., 2017).

Plecanatide

An oral GC-C agonist, plecanatide, consists of a 16-amino acid synthetic peptide equivalent to human uroguanylin and is used to treat gastrointestinal (GI) disorders. For the treatment of chronic idiopathic constipation (CIC) in adults, plecanatide received its first global approval in 2017 (Al-Salama and Syed, 2017; Rao, 2018). Plecanatide metabolism occurs in the gastrointestinal tract. After oral administration of 3 mg of plecanatide, blood levels of plecanatide and its active metabolites were lower than detectable levels. Standard pharmacokinetic parameters cannot be calculated, and the amount of plecanatide or its metabolites in the tissue is negligible due to the small amount of drug absorbed (Miner, 2020).

In two large randomized, double-blind, placebo-controlled studies evaluating the efficacy and safety of plecanatide (3 mg, 6 mg) vs placebo in patients with CIC, plecanatide treatment also significantly reduced the severity of other CIC symptoms (tension, consistent stools, bloating). In addition, the satisfaction and quality of life of patients treated with plecanatide improved significantly. The low incidence of adverse reactions after plecanatide treatment shows that it is safe and effective for CIC. In addition, plecanatide combined with acid suppressants is safe and effective in patients with CIC (DeMicco et al., 2017; Miner et al., 2017).

A meta-analysis evaluating the efficacy and tolerability of GC-C agonists included eight randomized controlled trials with 10,369 patients. Both drugs were effective in treating CIC, and the incidence of diarrhea was higher than that in the placebo group. Linaclotide and plecanatide were similar in efficacy and tolerability to IBS-C and CIC. There was no difference in the incidence of adverse reactions (diarrhea) between linaclotide and plecanatide (Shah et al., 2018).

Other medicines

Dolcanatide

Dolcanatide (SP-333), an oral uroguanylinoid, is replaced by selected D-amino acids to enhance stability and extend persistence, activating GUCY2C in the small and large intestines. A phase I double-blind, placebo-controlled trial (NCT03300570) of 27 mg dolcanatide administered orally daily for 7 days in healthy volunteers did not show activation of GUCY2C in distal rectal epithelial cells, as quantified by the accumulation of its product cGMP. These data suggest that the high stability of dolcanatide and its persistence along the rostral–caudal axis of the small and large intestines are insufficient to regulate GUCY2C throughout the colorectal region to prevent tumorigenesis. These results highlight the importance of developing GUCY2C anticancer agonists that target colorectal release and activity (Weinberg et al., 2021). Current research on dolcanatide focuses on its potential use for the treatment of colon cancer.

Summary

GC is widely distributed throughout the human body, mostly as sGC and GC-C, which catalyze GTP and thus increase the intracellular content of the second messenger cGMP, leading to the modulation of various intracellular physiological regulatory processes. The development of drugs on the basis of functional modulation of the GC pathway has been a fast-moving area of research over the past few years. A series of clinical studies have validated the therapeutic potential of sGC stimulators in patients with HF and PAH. Due to promising clinical trial results, riociguat and vericiguat have been approved by the US FDA for the treatment of chronic heart failure and pulmonary hypertension. In addition, the role of sGC stimulants in improving cGMP signaling might enable them to play an active role in a wide range of clinical indications, such as metabolic diseases, fibrotic diseases, urinary diseases, and neurodegenerative diseases. However, clinical studies have

shown that the benefit for dcSSc patients and patients with diabetic nephropathy is debatable, and more clinical studies are needed to support its use or nonuse in them. In contrast to sGC stimulants, the treatment potency of sGC activators is not fully clarified, although they can bind to sGC when the body is in a disease condition involving oxidative stress. The development of this class of drugs is still at the clinical study stage. In intestinal epithelial cells, GC-C is the dominant supplier of cGMP. The GC-C molecule is important for maintaining fluid and ion homeostasis in the intestinal tract, and linaclotide and plecanatide are the main drugs targeting it. Preclinical and clinical evidence shows that modulation of GC-C may improve symptoms and be tolerated by patients with IBS-C and CIC. Recent studies have shown that GC-C is also relevant to intestinal inflammation, dysbiosis and cancer, the specific mechanisms of which remain to be explored. In conclusion, cGMP has a broad spectrum of physiological effects, which gives it considerable development prospects. We systematically reviewed the transduction procedures of the NO-sGC-cGMP signaling pathway and the potential use of sGC stimulators and GC-C stimulators. Novel compounds are being developed based on the structures of sGC and GC-C, some of which are being studied in clinical trials. These clinical results may open up new therapeutic approaches for cardiovascular, renal and other diseases. Various questions remain regarding the mechanisms of their effects and the therapeutic potential of these treatments for diseases besides PAH and HF, and much more research is needed in the future.

Author contributions

QY: Writing–original draft, Writing–review and editing. XZ: Writing–original draft, Writing–review and editing. YS: Writing–original draft. LW: Drew the figures and tables, Writing–original draft. LL: Drew the figures and tables, Writing–original draft. RT: Writing–review and editing. LH: Writing–review and editing. YB: Supervision, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

GTP	guanosine triphosphate
cGMP	cyclic guanosine monophosphate
sGC	soluble guanylate cyclase
NO	nitric oxide
CO	carbon monoxide
H-NOX	N-hemoglobin-nitric oxide
GC-C	guanylate cyclase C
ECD	extracellular domain
NOS	nitric oxide synthases
eNOS	endothelial nitric oxide synthase
nNOS	neuronal nitric oxide synthase
PKGs	protein kinases
PDEs	phosphodiesterases
PLB	phospholamban
RyR2	Cardiac ryanodine receptor
SERCA	sarco endoplasmic reticulum Ca2+-ATPase
ER	endoplasmic reticulum
LTCCs	L-type calcium channels
TRPC6	transient receptor potential canonical channel type 6
TnI	troponin I
TTN	titin
CMyBP-C	cardiac myosin-binding protein-C
VSMC	venous smooth muscle cell
MLCP	myosin light chain phosphatase
RhoA	Ras homolog family member A
RGS-2	regulator of G-protein signaling 2
IP3	inositol 1,4,5-trisphosphate
Rap1Gap2	Rap1 GTPase-activating protein 2
Rap1b	Ras-related protein 1
IRAG	IP3-induced calcium release
GRP2	guanyl-releasing protein 2
BKCa	calcium-sensitive potassium channels
PASMCs	pulmonary artery smooth muscle cells
MLCK	myosin light chain kinase
VASP	vasodilator-stimulated phosphoprotein
BMP	bone morphogenetic protein
SMAD	small mothers against decapentaplegic
PDE5	phosphodiesterase 5
NFAT	calcineurin-nuclear factor of activated T cells
HF	heart failure
CVD	cardiovascular disease
HFrEF	heart failure with reduced ejection fraction
HFpEF	heart failure with preserved ejection fraction
ERK	extracellular signal-regulated kinase
ECM	extracellular matrix
CKD	chronic kidney disease
PAH	pulmonary arterial hypertension
CTEPH	chronic thromboembolic pulmonary hypertension
AE	adverse event
SAE	serious adverse event
RV	right ventricular
PH-IIP	pulmonary hypertension associated with idiopathic interstitial pneumonia
6MWD	6-min walk distance
PAP	pulmonary artery pressure
PVR	pulmonary vascular resistance
RAP	right atrial pressure
CI	cardiac index
CO	cardiac output
BPA	balloon pulmonary angioplasty
SAPH	sarcoidosis associated pulmonary hypertension
PK	pharmacokinetics
WHO-FC	World Health Organization Functional Classification
TAC	transverse aortic constriction
LVEF	left ventricular rejection fraction
LVM/BW	left ventricular mass to body weight ratio
MYH7	myosin heavy chain 7
PLN	cardiac phosphoprotein
ANKRD1	ankyrin repeat domain-containing protein 1
dcSSc	diffuse cutaneous systemic sclerosis
mRSS	modified Rodnan skin score
HUVECs	human umbilical vein endothelial cells
PACs	pro-angiogenic cells
CCSs	chronic coronary syndromes
BP	blood pressure
SBP	systolic blood pressure
DBP	diastolic blood pressure
PD	pharmacodynamics
ECG	electrocardiogram
DN	diabetic nephropathy
RPTC	renal proximal tubular epithelial cells
T2D	type 2 diabetes
RHI	reactive hyperemia index

HLI	hind limb ischemia
ICAM1	intercellular adhesion molecule 1
Myh2	MyHCIIa
Cxcl12	C-X-C motif chemokine ligand 12
PEG	polyethylene glycol
SSc-MVECs	SSc dermal microvascular endothelial cells
RBCs	red blood cells
TGFβ	transforming growth factors β
CYP450	cytochrome P450
cGKI	cGMP-dependent protein kinase type I
CLb	blood clearance
uHTS	ultrahigh-throughput screening
PCWP	pulmonary capillary wedge pressure
ADHF	acute decompensated heart failure
IOP	intraocular pressure
CAVS	calcific aortic valve stenosis
SMA	smooth muscle actin
PRP	platelet rich plasma
SRM	sirius red morphometry
DKD	diabetic kidney disease
CFTR	cystic fibrosis transmembrane regulator
CI-	chloride ions
cAMP	cyclic adenosine monophosphate
PKA	protein kinase A
HCO3-	bicarbonate ion
NHE3	Na+/H+ exchanger 3
IBDs	inflammatory bowel diseases
MRP4	multidrug resistance-associated protein 4
Akt	protein kinase B
p38 MAPK	p38 mitogen-activated protein kinase
CNGs	cyclic nucleotide-gated ion channels
MMP-9	matrix metalloproteinase-9
IBS-C	irritable bowel syndrome with constipation
CC	chronic constipation
CSBM	complete spontaneous defecation
IBS-SSS	IBS Symptom Severity Scale
IBS-QOL	IBS Quality of Life Questionnaire
CI	confidence interval
FC	functional constipation
GI	gastrointestinal
OIC	opioid-induced constipation
SBMs	spontaneous bowel movements
CIC	chronic idiopathic constipation
AUC	area under the concentration-time curve
Cmax	maximum concentration
T1/2	drug half-life
GUCY2C	Guanylate cyclase C
TEAEs	treatment-emergent adverse events
IIP	Idiopathic Interstitial Pneumonias
NT-proBNP	N-terminal pro-B-type natriuretic peptide
LVEDV	left ventricular end-diastolic volume
LVESV	left ventricular end-systolic volume
ABPM	Ambulatory BP monitoring
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
ADOs	adverse events resulting in study drug discontinuation
BFT	Supine Bolus Flow Time
VAS	visual analog scale
LOCF	last observation carried forward
NPSI	Neuropathic Pain Symptom Inventory
PAD	Peripheral Arterial Disease
ICD	initial claudication distance
AVC	Aortic Valve Calcification
UACR	urinary albumin-to-creatinine ratio
NPDR	nonproliferative diabetic Retinopathy
DRSS	Diabetic Retinopathy Severity Scale
CSPH	clinically significant portal hypertension
HVPG	hepatic venous pressure gradient
CTCAE	Common Terminology Criteria for Adverse Events
FVC	forced vital capacity
KCCQ-CSS	Kansas City Cardiomyopathy Questionnaire Clinical Summary Score
EQ-5D	5-dimension EuroQol questionnaire
LAV	left atrial volume

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EDITED BY
Ismail Laher,
University of British Columbia, Canada

REVIEWED BY
Bisher Abuyassin,
King Abdullah International Medical Research Center (KAIMRC), Saudi Arabia
Keliang Xie,
Tianjin Medical University, China

*CORRESPONDENCE
Jianhua Li,
jianhuali2022@cqu.edu.cn

†These authors have contributed equally to this work

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The pathogenesis and therapeutic strategies of heat stroke-induced myocardial injury

Rui Xia[1†], Meng Sun[2†], Yuling Li[3], Jing Yin[4], Huan Liu[1], Jun Yang[1], Jing Liu[1], Yanyu He[1], Bing Wu[1], Guixiang Yang[1] and Jianhua Li[1*]

[1]Department of Critical Care Medicine, Chongqing University Jiangjin Hospital, Chongqing, China, [2]Department of Anesthesiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [3]Emergency Department, The First Affiliated Hospital of Dalian Medical University, Dalian, China, [4]Nanjing Jinling Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, China

Heat stroke (HS) is a febrile illness characterized by an elevation in the core body temperature to over 40°C, accompanied by central nervous system impairment and subsequent multi-organ dysfunction syndrome. In recent years, the mortality rate from HS has been increasing as ambient temperatures continue to rise each year. The cardiovascular system plays an important role in the pathogenesis process of HS, as it functions as one of the key system for thermoregulation and its stability is associated with the severity of HS. Systemic inflammatory response and endothelial cell damage constitute pivotal attributes of HS, other factors such as ferroptosis, disturbances in myocardial metabolism and heat shock protein dysregulation are also involved in the damage to myocardial tissue in HS. In this review, a comprehensively detailed description of the pathogenesis of HS-induced myocardial injury is provided. The current treatment strategies and the promising therapeutic targets for HS are also discussed.

KEYWORDS

heat stroke, myocardial injury, pathogenesis, therapeutic strategy, inflammation

1 Introduction

Heat stroke (HS) is an illness characterized by a rapid rise of core temperature over 40°C with the complication of systemic inflammatory responses and central nervous system dysfunction (Bouchama and Knochel, 2002; Leon and Helwig, 2010; Peiris et al., 2017). In recent years, heat-related deaths have increased significantly due to anthropogenic climate change (Toutant et al., 2011). Frequency of severe heat waves is threatening human health worldwide and poses huge challenges to public health, attracting widespread attention in various research fields (M. Zheng et al., 2020). HS can be divided into classic heat stroke (CHS) and exertional heat stroke (EHS) depending on the involvement of skeletal muscle contraction (Bouchama et al., 2022). CHS often occurs in older people having pre-existing illnesses, while EHS typically occurs in healthy younger individuals during strenuous

Abbreviations: HS, Heat stroke; CHS, Classic heat stroke; EHS, Exertional heat stroke; SIRS, Systemic inflammatory response syndrome; TNF-α, Tumor necrosis factor-alpha; IL-1β, Interleukin-1β; NLRP3, NOD-like receptor family pyrin domain containing3; vWF, von Willebrand factor; DIC, Disseminated intravascular coagulation; ROS, Reactive oxygen species; GSH, Glutathione; SLC7A11, Solute carrier family 7 member 11; MDA, Malondialdehyde; ACC, Acyl CoA-carboxylase; FAs, Fatty acids; GLUT4, Glucose transporter 4; HSP, Heat shock protein.

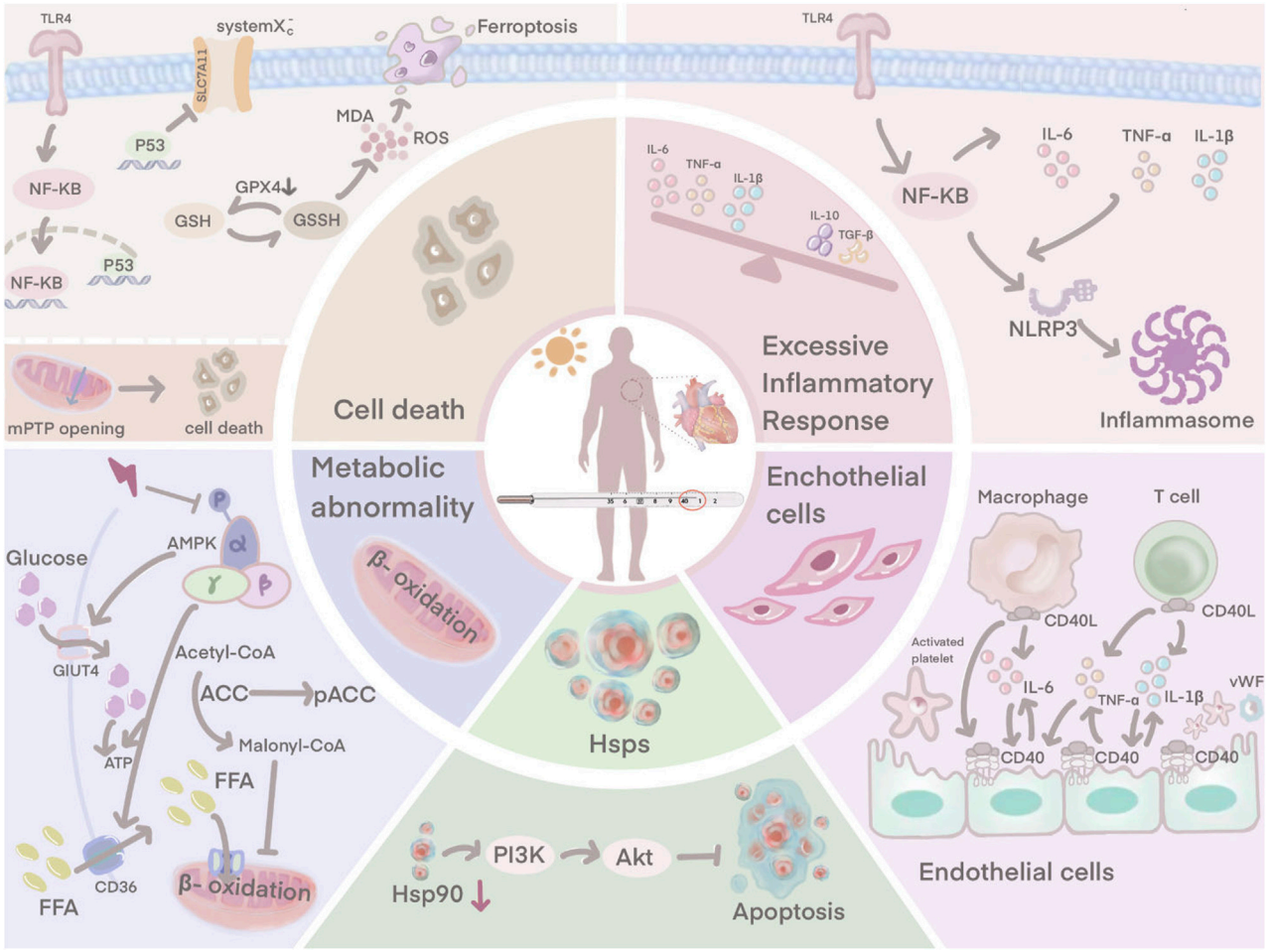

FIGURE 1
HS-induced myocardial injury is not only associated with an excessive inflammatory response, endothelial cell damage. Ferroptosis, downregulation of HSP90 expression and disturbances in cardiomyocyte metabolism are also involved.

exercise in hot environments (Peiris et al., 2017; Bouchama et al., 2022). HS, regardless of the type, is associated with extensive multi-organ tissue damage as a result of the interaction of cytotoxic, inflammatory, and clotting reactions (Piver et al., 1999). The heart, being a vulnerable organ in heat injury (Low et al., 2011; Lou et al., 2019; Ko et al., 2020), is susceptible to arrhythmia, function failure and focal myocardial necrosis (Argaud et al., 2007; Desai et al., 2023).

Abnormalities in temperature regulation, cardiovascular function and tissue perfusion are among the factors involved in multiple organ dysfunction syndrome (Low et al., 2011; Cramer et al., 2022). In an effort to dissipate heat, the body increases blood flow to the skin, redistributes blood and eventually develops hypotension and perfusion disorders (S. H. Chen et al., 2006). Thus, the regulation of the cardiovascular system plays a key role in the pathogenesis of HS. Elucidation of the mechanism of HS-induced myocardial injury can help in establishing the treatment to improve circulatory function and reduce mortality rates of HS. However, the pathogenesis of HS is still to be known and prevention strategies of myocardial injury during HS is lacking. This article provides a systematic review to further the understanding of HS-induced myocardial injury and to provide a reference for future research (Figure 1).

2 Heat stroke and myocardial injury

Under normal conditions, a 0.3°C increase in core temperature triggers a cardiovascular regulatory response to protect the body from heat damage. This regulation increases heat dissipation by speeding up the heart rate, enhancing cardiac contractility, raising cardiac output and reducing blood flow and volume in non-skin areas (Crandall et al., 2008; Crandall and González-Alonso, 2010).

During HS, when the surrounding hot environment persists, the above regulation continues to function actively. A substantial volume of blood is pumped from the heart towards the peripheral blood vessels to dissipate heat through sweat, but this also results in hyperthermic dehydration of the body, reduced circulating blood volume, inadequate tissue perfusion, hypoxia and necrosis of myocardial cells (Crandall and González-Alonso, 2010; G. D; Chen et al., 2019). At the same time, the loss of body fluids disturbs electrolytes and interrupts the sodium-potassium

pump, which alters the heart's pacing rhythm, signal conduction and systolic-diastolic functional state, ultimately leading to myocardial ischemia, necrosis, arrhythmia and heart failure (Hausfater et al., 2010; Chen, et al., 2019; Tseng et al., 2019; Wang et al., 2019).

3 The related mechanisms of HS-induced myocardial injury

3.1 Dysregulation of the pro-inflammatory and anti-inflammatory balance

In HS, the systemic pro- and anti-inflammatory balance is disturbed, triggering a systemic inflammatory response syndrome (SIRS) that is thought to be characteristic (Epstein and Yanovich, 2019). The pathogenesis of heat stroke is closely similar to that of sepsis (Roberts et al., 2008). In a hot environment, the dilatation of blood vessels on the body surface due to heat dissipation leads to reduced blood flow to internal organs, especially intestinal mucosa, which causes increased intestinal epithelial permeability and bacterial translocation in the intestine, inducing leakage of intestinal endotoxins through the intestine into the circulation and triggering SIRS, ultimately leading to multi-organ dysfunction and death (Yang et al., 2007; Lambert, 2008; Leon and Helwig, 2010). The systemic inflammation associated with heat stroke plays a key role in myocardial injury. Currently, it is thought that the myocardial inflammatory response may be the primary cause of progressive systolic dysfunction (Dörge et al., 2000; Dörge et al., 2002). A large infiltration of inflammatory cells is usually found within the foci of myocardial infarction. Previous studies have shown that suppression of the inflammatory response is an important tool in the treatment of HS-induced myocardial injury (Lin et al., 2017; Lin et al., 2018; Lin et al., 2020).

During HS, the body undergoes a state of hypercytokinemia, releasing many cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) (Leon and Helwig, 2010; Z. T; Zhang et al., 2021). TNF-α, a key factor in the inflammatory response, plays an important role in neutrophil recruitment and the inflammatory cascade reaction (Yu et al., 2010). In addition, TNF-α induces the production of other inflammatory cytokines and also stimulates the migration and adhesion of neutrophils, leading to dysregulation of pro- and anti-inflammatory factors and inducing an inflammatory cascade reaction, which results in tissue damage (Yu et al., 2010). At the same time, the injured myocardial tissue also releases pro-inflammatory cytokines, including TNF-α and IL-6, which further exacerbate the systemic inflammatory response (Shen et al., 2019).

The TLR4/NF-κB signaling pathway has a major contribution to HS-induced inflammation. TLR4 is an essential member of the TLR family and plays a central role in the recognition and response to microbial pathogens and in maintaining the integrity of the intestinal epithelial barrier (D. Yao et al., 2019). Rats subjected to heat stress have significantly elevated levels of TLR4 (D. Chen et al., 2023). When rats are affected by heat stress, NF-κB is activated by the induced TLR4, leading to the release of pro-inflammatory factors. The production and release of pro-inflammatory factors further activates NF-κB, which induces the NLRP3 inflammasome, leading to a sustained amplification of the initial inflammatory signal, thus causing the so-called inflammatory cascade effect (Z. Huang et al., 2016; X; Zhang et al., 2017). TLR4 exhibits its highest expression in cardiac myocytes, and during HS, TLR4/NF-κB signaling controls the production of pro-inflammatory factors to induce myocardial tissue damage (X. Liu et al., 2016). Inhibition of the TLR4 signaling pathway may reduce HS-induced inflammatory responses and improve abnormal cardiac function in rats (Chen et al., 2023).

3.2 Endothelial cell damage and dysfunction

Cardiac ultrastructure in HS patients exhibits severe endothelial cell damage (Sohal et al., 1968). Vascular endothelial cells cover the surface of the lumen and maintain the structural integrity and microcirculatory function of the coronary microvasculature (Chang et al., 2021). It also acts as a defensive barrier against the penetration of microorganisms, immune cells and coagulation components, which reduces the risk of thrombosis (Chang et al., 2021). Activated *in vivo* crosstalk exists between vascular endothelium, inflammation and coagulation during HS (Bouchama et al., 1991; al-Mashhadani et al., 1994; Roberts et al., 2008). Endothelial cell dysfunction plays a key role in the initiation and progression of HS (W. Huang et al., 2022).

Endothelial cells possess an anti-inflammatory effect under normal physiological conditions, repelling circulating neutrophils from adhesion (Chang et al., 2021). However, when rat myocardial tissue is damaged by heat stress, endothelial cells upregulate a variety of adhesion molecules that attract pro-inflammatory cells (neutrophils and macrophages) to secrete pro-inflammatory cytokines (Harlan et al., 1991; Wihastuti et al., 2018; Chang et al., 2021). Large amounts of pro-inflammatory factors such as IL-6 and TNF-α can trigger endothelial dysfunction and microvascular damage (F. Chen et al., 2017). Damaged endothelial cells express CD40, and in the presence of CD40 interacting with CD40 ligand (CD40L), endothelial cells actively secrete von Willebrand factor (vWF), which promotes platelet adhesion to endothelial cells and contributes to thrombosis (Keuren et al., 2004; Han et al., 2018). The interaction between CD40 and CD40L also stimulates platelets and endothelial cells to activate macrophages and T cells, which further amplifies the inflammatory response (Urbich et al., 2002). Damage to the endothelium, a natural barrier against thrombosis, upregulates procoagulant factors and downregulates anticoagulant factors, thereby disturbing the dynamic balance between pro- and anti-thrombotic activities and inducing microthrombosis (Koupenova et al., 2017). Obstruction of small vessels contributes to infarction and necrosis of myocardial tissue. The damaged tissue releases plasminogen activator which induces the development of disseminated intravascular coagulation (DIC) (Sohal et al., 1968). Hearts of patients with HS show evidence of extensive visual and microscopic haemorrhage (Sohal et al., 1968).

Aspirin, a non-steroidal anti-inflammatory drug, that not only inhibits platelet aggregation but also maintains the integrity of endothelial gap junctions (Zhou et al., 2019). Animal study has shown that the treatment with aspirin significantly improves the morphological damage and related enzyme activity of chicken cardiomyocytes induced by heat stress (Wu et al., 2016).

3.3 Abnormal cardiomyocyte death

HS instigates multiple toxic effects on the cardiovascular system, including abnormal cardiomyocyte death (Chen et al., 2017; Chen et al., 2023). The damaged myocardial cells exhibit vacuolar changes and partial necrosis (Fan et al., 2015; Chen et al., 2019). Ferroptosis is an essential form of abnormal cardiomyocyte death caused by HS, resulting from the excessive accumulation of iron-dependent lipid reactive oxygen species (ROS) in cells, where lipid peroxidation is a key component in triggering ferroptosis (Del Re et al., 2019; Stockwell et al., 2020). HS disrupts the oxidation-antioxidant balance, as evidenced by a decrease in glutathione (GSH) and solute carrier family 7 member 11 (SLC7A11), an increase in malondialdehyde (MDA), ROS, and Fe^{2+}. HS also induces shrinkage of mitochondria and an increase in the membrane density, which are key features of ferroptosis (Jiang et al., 2021; Chen et al., 2023). This suggests that ferroptosis is actively involved in HS-induced myocardial injury and causes abnormal cardiomyocyte death. Chen et al. further explored the mechanism of ferroptosis in the HS myocardial injury model (D. Chen et al., 2023). P53 expression levels were closely associated with the triggering of ferroptosis (Lei et al., 2021), and its involvement as a transcriptional repressor of SLC7A11 to ferroptosis significantly reduced the expression of SLC7A11, which in turn inhibited the activity of system X_c^-, a component of SLC7A11 (Koppula et al., 2018), thereby inhibiting cysteine uptake and reducing GPX4 activity leading to depletion of GSH biosynthesis (Xu et al., 2021; Zhang et al., 2022). Consequently, lipid peroxide accumulation ensued, ultimately culminating in cellular ferroptosis (Ma et al., 2022). P53, one of the molecules downstream of TLR4, is activated by the TLR4/NF-κB signaling pathway, which plays an active role in the systemic inflammatory response induced by HS (Zhu et al., 2011). In view of this, Chen et al. suggested that HS may induce ferroptosis through the TLR4/NF-κB/P53 signaling pathway (Chen et al., 2023). Inhibition of TLR4 and NF-κB under HS conditions downregulated P53 expression, upregulated SLC7A11 and GPX4 levels, improved ferroptosis-related indicators and attenuated myocardial injury, respectively (Chen et al., 2023).

Disruption of mitochondrial structure and function can lead to severe cellular damage and death (Zamzami et al., 1997; D'Orsi et al., 2017). Mitochondria plays a crucial role in maintaining intracellular calcium homeostasis (D'Orsi et al., 2017). From rat cardiomyocytes, we know that heat stress causes mitochondrial changes in cardiac myocytes including mitochondrial swelling, rupture of cristae and disruption of the surrounding membrane (Petit et al., 1998; Qian et al., 2004). Ca^{2+}-ATPase on the mitochondrial membrane serves as critical factor in the regulation of calcium homeostasis. However, disruption by heat stress leads to a decrease in Ca^{2+}-ATPase activity, which results in reduced mitochondrial uptake of calcium ions from the cytoplasm and intracellular calcium overload (McCormack and Denton, 1989; Walkon et al., 2022). Intracellular calcium overload further activates calcium-dependent protein kinases, which promote membrane phospholipid hydrolysis, disrupting the cytoskeleton and damaging the integrity of the nucleus, causing severe damage (Vassalle and Lin, 2004). HS directly induces the opening of mitochondrial mPTP, a pivotal event in triggering the cell death pathway (Halestrap, 2009; Bauer and Murphy, 2020). mPTP opening results in a series of cytological effects that lead to the release of cytochrome c, activation of caspase family proteases and apoptosis of cardiomyocytes (H. Yao et al., 2022). The mechanism by which HS induces mPTP opening is not yet clear, and the Fas pathway is an important signaling pathway to consider. It induces caspase-8 activation, which subsequently directly activates caspase-3 and leads to the opening of mPTP (Nakamura et al., 2000). However, whether the Fas pathway is involved in HS-induced mPTP opening remains to be explored.

3.4 Metabolic abnormalities

The link between metabolic dysregulation and cardiotoxicity has been well established (Russo et al., 2021). Mitochondrial damage caused by HS not only results in abnormal death of cardiomyocytes but also leads to disturbances in energy metabolism (Azevedo et al., 2013). Energy abnormalities in the heart are associated with the development of many heart diseases (X. Wang et al., 2023). Heat stress disrupts the integrity of the mitochondria, which is the basis for normal mitochondrial function, resulting in a suppression of energy production from the oxidative metabolism of cardiomyocytes (Patra and Hay, 2014; Laitano et al., 2020; Deng et al., 2022). However, in response to the high temperatures of the external environment, the heart requires a greater supply of energy to enhance cardiac function, which leads to a significant decrease in the ATP content of the cardiomyocytes and eventual death due to energy deficiency (Qian et al., 2004).

Glucose and fatty acids are essential substrates for oxidative phosphorylation. Glucose and lipid metabolism plays an important role in cardiac myocytes by providing energy and maintaining cellular function (H. Tian et al., 2023). However, studies in murine models of EHS have revealed that HS alters cardiomyocyte metabolic pathways, disrupts the glycolytic and oxidative phosphorylation pathways by upregulating glycolysis-related enzymes, thereby enhancing lactate production to impair cardiomyocyte function (Laitano et al., 2020). The perturbation of glucose and lipid metabolism by HS may be related to the inhibition of the AMPK signaling pathway (Rodríguez et al., 2021). AMPK increases ATP production in cardiomyocytes through stimulation of glucose metabolism and fatty acid oxidation. AMPK phosphorylation at Thr^{172} induces acyl CoA-carboxylase (ACC) phosphorylation to inhibit the conversion of acetyl-CoA to malonyl-CoA during fatty acids (FAs) synthesis (Carling et al., 2008). Beyond the inhibition of lipid anabolism, p-AMPK also promotes FAs uptake by inducing the activity of the FAs transporter CD36, enhancing β-oxidation (Habets et al., 2009). Glucose metabolism is also regulated by AMPK. p-AMPK increases glucose transporter 4 (GLUT4), which promotes glucose uptake and thus provides a source of energy (D. Zheng et al., 2001). Under HS conditions, phosphorylation of AMPK is inhibited, leading to dysregulation of glucolipid metabolism and disruption of energy metabolism (Roths et al., 2023). This ultimately leads to cell death and impaired cardiac function. Therefore, targeting glucose and lipid metabolism may be an effective way to counteract HS-induced myocardial injury.

3.5 Heat shock protein dysregulation

Cells from a murine model of myocardial tissue turn on their intrinsic defense mechanisms in the face of heat injury, with a dramatic increase in heat shock protein (HSP) expression being a key part of the heat shock response (Tang et al., 2013; Tang et al., 2016). It can interlock with apoptosis, inflammation and autophagy to regulate cellular homeostasis and prevent tissue damage (Hsu et al., 2013; Shen et al., 2019). It was mentioned earlier that patients with HS can develop severe vascular endothelial cell damage. After heat exposure, strong positive signals for HSP90 and HSP70 are detected in rat cardiac microvascular endothelial cells, helping the vascular endothelium to resist heat injury (X. Zhang et al., 2020). An increase in HSP90 activates the PI3K/Akt signaling pathway. Phosphorylated Akt negatively regulates the expression of pro-apoptotic proteins and contributes to cell survival (Zhang et al., 2020). It is known from rat-related experiments that HSP levels vary with the duration of heat stress. In the early stages of HS, HSP rises sharply, and as time progresses, HSP is heavily depleted, resulting in abnormally low HSP levels in the later stages (H. B. Chen et al., 2015; Lin et al., 2020). When HSP90 is crushed, the interaction of HSP90 with Akt is reduced, weakening the protective effect. This results in the vascular endothelium exhibiting a more sensitive state to heat stress and more severe damage (Zhang et al., 2020).

4 The treatment strategy for HS

The prognosis of patients with heat stroke is directly related to the degree and duration of the increase in core temperature (Hadad et al., 2004). Therefore, whole-body cooling is the current treatment of choice for HS. Following the onset of HS, hypotension and altered cardiac protein profiles are demonstrated, which can be reversed by whole-body cooling (Ko et al., 2020). Temperature reduction is achieved mainly by conduction, evaporation and convection (Hadad et al., 2004). In addition, symptomatic support therapy is an integral part of the treatment. When hypotension occurs in patients, aggressive fluid resuscitation and vasoactive medication should be administered with the avoidance of alpha-adrenergic drugs as they exacerbate peripheral vasoconstriction and inhibit core body temperature reduction (Atha, 2013; Asmara, 2020). Excessive inflammation and coagulation disorders are important pathogenic mechanisms of HS, therefore anti-inflammatory and anticoagulant therapies are also available as treatment options (Y. F. Tian et al., 2013; Kobayashi et al., 2018). For patients who progress to multi-organ dysfunction despite hypothermia treatment, continuous blood purification and plasma exchange are often selected, aiming not only to alleviate the body's catabolic state but also to eliminate inflammatory mediators from the bloodstream to facilitate the recovery of HS patients (Wakino et al., 2005; K. J; Chen et al., 2014). Given the danger and intractability of HS, prevention strategies are far more beneficial than any present treatment strategies. People at risk of heat exposure should be thermally acclimatized in advance, with consumption of sufficient fluids and adequate nutrition (Asmara, 2020).

5 Discussion

HS involves a complex biochemical cascade of reactions and is caused by a combination of factors. The cardiovascular system is considered to be the first system affected by HS. Circulatory shock occurs in approximately 20%–65% of patients, and an even higher 85% of patients will develop ECG abnormalities (Austin and Berry, 1956; Asmara, 2020). However, a dearth of clinical directives exists regarding the efficacious management of cardiovascular ailments amidst elevated temperatures. A precise comprehension of the fundamental mechanisms whereby heightened temperatures inflict harm upon myocardial tissue is imperative to judiciously formulate preventative and therapeutic strategies. This review summarizes the possible pathogenesis of HS-induced myocardial injury, which can help provide new targets for the treatment of HS.

The predominant body of research scrutinizing myocardial impairment due to hyperthermia predominantly comprises animal studies, with a paucity of involvement from clinical cohorts. The acquisition of clinical data assumes heightened significance. A comprehensive database analysis encompassing 27 countries spanning the years 1979–2019 revealed a 7% escalation in mortality among patients with ischemic heart disease during episodes of soaring temperatures (Alahmad et al., 2023). Moreover, a meta-analysis delineated a 2.8% augmentation in the risk of developing coronary heart disease for each 1°C ascent in temperature (J. Liu et al., 2022). Endothelial cell damage within cardiac vasculature due to pyrexia precipitates thrombosis, culminating in acute coronary incidents. Clinical investigations have documented a substantial surge in hospitalizations linked to coronary artery disease following exposure to elevated temperatures (Fuhrmann et al., 2016). Long-term monitoring of hyperthermia-stricken patients corroborates the critical role of intact myocardial tissue, with a meager 1-year survival rate of merely 24% observed in cases with markedly elevated troponin levels (Marchand and Gin, 2022). This underscores the profound impact of myocardial impairment on the prognosis of hyperthermia-afflicted individuals. Consequently, the primary focus of research should pivot towards averting myocardial damage induced by HS.

The incomplete comprehension of HS pathogenesis, coupled with the absence of evidence-based medical guidance for clinical interventions, has resulted in the inadequacies of current treatment modalities. Predominantly, supportive therapies such as whole-body cooling and fluid resuscitation constitute the primary approach. Regrettably, a lack of standardized endpoint objectives for whole-body cooling persists to date. Furthermore, despite numerous animal studies affirming the favorable efficacy of anti-inflammatory and anti-endotoxic agents for HS, their translation into clinical success remains limited. Aspirin, despite demonstrating effectiveness against heat-induced injury in avian cardiac tissues, fails to manifest any clinical benefit and may potentially exacerbate coagulation disorders and hepatic dysfunction (Tek and Olshaker, 1992). Individuals with cardiovascular ailments not only contend with the vulnerability of their cardiac systems in the face of HS but also grapple with an elevated risk due to commonly prescribed cardiac medications. β-blockers impede the capacity to augment cardiac output in response to HS, while diuretics exacerbate hypovolemia and elevate the risk of electrolyte imbalances (Marchand and Gin, 2022). This begs the question of which

medications can be initiated or stopped during extreme heat conditions.

In cases where the heart receives heat damage, significant changes in cardiac metabolism occur. These changes are not only passive bystanders, but are actual participants in causing heat stress damage to the myocardium. Targeting myocardial metabolism could be the tool for our effective treatment. Interventions in cardiac metabolic processes have been successfully used to reduce infarct size in animal models of myocardial ischaemia-reperfusion injury (Valls-Lacalle et al., 2018; Zuurbier et al., 2020). However, suitable drug targets for conversion in patients with acute myocardial infarction are still awaited. Cardiometabolic therapies are challenging, but fortunately, recent methodological advances in detecting metabolic changes within the heart will make our efforts more achievable.

In conclusion, HS-induced myocardial injury arises from a combination of excessive inflammation, endothelial cell damage, abnormal cardiac metabolism, and heat shock protein dysregulation. In the treatment, in addition to systemic supportive therapy it should also focus on precise targeting of myocardial tissue. Only with a deeper and clearer understanding of the mechanisms underlying the development of HS will there be an opportunity to establish more effective treatment.

6 Future perspective

Given the increasing mortality rate associated with HS, extensive research has been conducted to explore this condition. A meticulous examination of the literature has revealed potential molecular targets for HS treatment, encompassing TLR4, P53, AMPK, and HSP. Additionally, the Fas signaling pathway presents a novel avenue for HS management. However, the majority of these investigations have been confined to the realm of animal studies, and the therapeutic strategies delineated await clinical validation. Consequently, we should focus more on clinical trials to find relevant drug targets that can serve clinical HS patients. Furthermore, the absence of targeted therapy for HS-induced myocardial injury underscores the need for advancements in this area. Fortunately, the rapid development of modern bioinformatics technologies offers us valuable tools to deepen our understanding of the pathogenesis of HS-induced myocardial injury and implement precise treatments.

Author contributions

RX: Writing–original draft, Writing–review and editing. MS: Writing–original draft. YL: Writing–review and editing. JY: Writing–review and editing. HL: Software, Visualization, Writing–review and editing. JY: Software, Visualization, Writing–review and editing. JL: Software, Writing–review and editing. YH: Visualization, Writing–review and editing. BW: Validation, Writing–review and editing. GY: Validation, Writing–review and editing. JL: Supervision, Writing–review and editing.

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EDITED BY
Ismail Laher,
University of British Columbia, Canada

REVIEWED BY
Qing Rex Lyu,
Chongqing General Hospital, China
Christian Aalkjaer,
Aarhus University, Denmark

*CORRESPONDENCE
Qingjian He,
18742590857@163.com

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Microvascular rarefaction caused by the NOTCH signaling pathway is a key cause of TKI-apatinib-induced hypertension and cardiac damage

WenJuan Wang[1], Guodong Li[1], Jie Ma[2], Xin Fan[2], Jianzhong Lu[1], Qiyin Sun[1], Jiafang Yao[1] and Qingjian He[3]*

[1]Department of Cardiovascular Center, The First People's Hospital of Huzhou City, Huzhou, China, [2]Department of Hypertension Center, Lanzhou University Second Hospital, Lanzhou, China, [3]Department of Breast and Thyroid Surgery, The First People's Hospital of Huzhou City, Huzhou, China

With the advancement of tumour-targeted therapy technology, the survival of cancer patients has continued to increase, and cardiovascular events have gradually become an important cause of death in cancer patients. This phenomenon occurs due to adverse cardiovascular reactions caused by the cardiovascular toxicity of antitumour therapy. Moreover, the increase in the proportion of elderly patients with cancer and cardiovascular diseases is due to the extension of life expectancy. Hypertension is the most common cardiovascular side effect of small molecule tyrosine kinase inhibitors (TKIs). The increase in blood pressure induced by TKIs and subsequent cardiovascular complications and events affect the survival and quality of life of patients and partly offset the benefits of antitumour therapy. Many studies have confirmed that in the pathogenesis of hypertension, arterioles and capillary thinness are involved in its occurrence and development. Our previous findings showing that apatinib causes microcirculation rarefaction of the superior mesenteric artery and impaired microvascular growth may inspire new therapeutic strategies for treating hypertension. Thus, by restoring microvascular development and branching patterns, total peripheral resistance and blood pressure are reduced. Therefore, exploring the key molecular targets of TKIs that inhibit the expression of angiogenic factors and elucidating the specific molecular mechanism involved are key scientific avenues for effectively promoting endothelial cell angiogenesis and achieving accurate repair of microcirculation injury in hypertension patients.

KEYWORDS

hypertension, microcirculation, rarefaction, TKIs, apatinib

1 Introduction

Hypertension has a high prevalence in China and accounts for a large proportion of cardiovascular diseases. Hypertension is also a major risk factor for cardiovascular-related diseases. In recent years, a number of studies have reported a relationship between tumours and hypertension. Tumours and hypertension have common risk factors and overlapping pathophysiological mechanisms (Milan et al., 2014; Bray et al., 2021; Dolmatova et al.,

TABLE 1 Incidence of hypertension caused by TKIs.

Drug	Category	Target spot	Hypertension (%)
axitinib	TKIs	VEGFR1-3,c-KIT,PDGFR	22–84
cabozantinib	TKIs	MET,VEGFR2,RET,AXL,FLT3	28–61
Lenvatinib	TKIs	VEGFR1-3, FGFR 1–4,PDGFR, c-KIT,RET	42–73
Pazopanib	TKIs	VEGFR1-3,PDGFR, FGFR,c-KIT	40–42
Ponatinib	TKIs	BCR-ABL,VEGFR, PDGFR,FGFR,EPH,c-KIT,RET,TIE2,FLT3	53–74
regorafenib	TKIs	VEGFR1-3,PDGFR,c-KIT,RET,RAF-1	28–67
Sorafenib	TKIs	VEGFR1-3,PDGFR,c-KIT,RET,RAF-1	4–31
sunitinib	TKIs	VEGFR2,PDGFR,c-KIT	20–27
Wandtaneb	TKIs	VEGFR2,PDGFR,c-KIT	4–40

2023). Therefore, several experts have gradually formed the theory of onco-hypertension. Previous studies by our group have shown that hypertension and antihypertensive drug use are closely related to breast cancer. With increasing age, the incidence of hypertension in breast cancer patients increases, and the mechanism is related to inflammatory mediators and angiogenesis (Zhao et al., 2018; Wang et al., 2021). Another reason is that women's estrogen levels decline as they age. Estrogen has a protective effect on blood vessels, so such patients are more likely to induce hypertension. And estrogen for breast cancer patients, with the increase of age, although estrogen in the body decreases, but some patients may be converted to androgen, leading to the increase in the incidence of breast cancer. In recent years, new antineoplastic drugs have prolonged the survival of cancer patients, but the increase in blood pressure caused by new antineoplastic drugs and the subsequent cardiovascular complications and events affect the survival and quality of life of patients, which partly offset the benefits of antineoplastic therapy. It is worth exploring this topic further.

2 Incidence of hypertension caused by TKIs

VEGF signalling pathway inhibitors include monoclonal antibodies against VEGF A factor, VEGF traps, monoclonal antibodies against VEGF receptors, and TKIs (Pucci et al., 2019; Le et al., 2021; Lawler, 2022). Studies have shown that the probability of hypertension caused by VEGF signalling pathway inhibitors during antitumour therapy is approximately 11%–45%, among which the incidence of hypertension above grade 3 (referring to CTCAE grade 3–4, that is, SBP≥160 and/or DBP ≥100 mmHg) or life-threatening hypertension, even requiring emergency treatment, ranges from 2 to 20% (Xu et al., 2021). According to the type and dose of TKIs, the incidence of hypertension can reach 20%–90%, and the incidence of severe hypertension can reach 6%–43% in patients using TKIs alone or in combination. The incidence of hypertension induced by TKIs is shown in Table 1.

Apatinib mesylate is an antitumour drug that was independently developed in China and belongs to the class of TKIs. In the past, it was mainly used for the treatment of solid tumours, such as advanced gastric cancer or gastroesophageal junction adenocarcinoma (Li et al., 2016). An international multicentre phase III study (SHR-1210-III.-310 study) demonstrated that the approved PD-1 inhibitor camrelizumab in combination with apatinib had a significant survival benefit and a tolerable safety profile in the first-line treatment of advanced liver cancer, with a median overall survival (OS) of 22.1 months. It is the combination therapy with the longest OS benefit in the first-line treatment of advanced liver cancer (Qin et al., 2023). Therefore, the National Medical Products Administration (NMPA) proposed the combination of "Shuang'ai" for the first-line treatment of advanced hepatocellular carcinoma, which is the first approval in China to use a combination of PD-1 inhibitors and TKIs for the treatment of advanced hepatocellular carcinoma. The application of this treatment regimen has led to new drug options for patients with advanced liver cancer. The resulting cardiovascular side effects of nitrogen are also very noteworthy. Therefore, studying the cardiovascular toxicity and side effects caused by TKIs is highly important for guiding antitumour therapy in clinical cancer patients.

3 Causal relationship between microcirculation damage and hypertension

3.1 Microcirculation and related definitions of microcirculation damage

Microcirculation typically includes small resistance arteries (300–100 μm in diameter), precapillary arterioles (100–10 μm), capillaries (5–15 μm) and venules (10–100 μm) (le Noble et al., 2023). Various studies have shown that damage to microcirculatory tissue may involve multiple mechanisms. The main categories of such damage include impaired endothelial cell function, oxidative stress, decreased angiogenesis (most commonly in patients after the use of targeted drugs), increased endothelial permeability, enhanced leukocyte adhesion, immune cell activation, lymphatic dysfunction, impaired autoregulation, microvascular constriction, and microcirculation obstruction (Sorop et al., 2020; Sabe et al., 2022; De Ciuceis et al., 2023; Mengozzi et al., 2023).

3.2 Microcirculation and types of microcirculation damage

Studies have shown a relationship between hypertension and changes in the microvascular network in spontaneously hypertensive rats (SHRs), with reduced arteriolar density and increased postcapillary venule density (Martens and Gelband, 1998). It lead to increased postvascular resistance, which may further contribute to the development of hypertension. In addition, it has been shown that a sparse microvascular network structure increases total peripheral resistance, which eventually leads to increased blood pressure (le Noble et al., 2023). Many previous studies have confirmed the importance of arteriolar and capillary scarcity in the pathogenesis of hypertension (Levy et al., 2001; Levy et al., 2008). That is, the rarefaction of capillaries may contribute to the development of hypertension.

Microcirculation injury can occur through two main forms of microvascular rarefaction (Agabiti-Rosei, 2003; Ungvari et al., 2021). Functional rarefaction means that the total number of anatomically present vessels is not reduced but rather that perfusion of this part of the microvascular network is absent. However, as the vascular tone continues to increase, the lumen area increases, resulting in a decrease in the number of blood vessels perfused. Structural sparseness also occurs, which reduces the number of blood vessels that can be found during tissue dissection. This reduction in vascular mesh may be due to altered anatomical changes in the vascular segments or other impairments in the vascular network during the growth and development process during early tissue development. Several experts have also noted that functional rarefaction can progress to structural rarefaction in a rat model of SHR. However, there are also studies showing that patients with essential hypertension, recruitment of perfused capillaries is impaired, which can be explained by both functional and structural rarefaction (Serné et al., 2001). Therefore, whether the microcirculation dilution in hypertension is structurally sparse or functional, or even both, will be our further research plan. This part of the study is crucial for them to identify the specific microcirculation sparse so that we can improve and treat it according to the cause, especially in patients with hypertension caused by targeted drugs.

3.3 Mechanisms of microcirculation damage leading to hypertension

Microvascular injury plays an indispensable role in the occurrence and development of hypertension, and some studies have shown that it plays an important role in the progression of hypertension, hypertension-mediated organ damage and related cardiovascular events (Jonk et al., 2007; Karaca et al., 2014; Rizzoni et al., 2023). However, the causal relationship between thinning of microvessels and hypertension is difficult to explain, and some experts speculate that diffuse generalized thinness of microvessels may be one of the main causes of hypertension (Marinescu et al., 2015; Horton and Barrett, 2021). In study, after 5 weeks of treatment with telatinib, the capillary density decreased from 20.8 at baseline to 16.7 (Steeghs et al., 2008). Mourad et al. reported a significant reduction in kinetic and structural capillary density in a group of patients with metastatic colon cancer treated with bevacizumab for 6 months (Mourad et al., 2008). However, this rarefaction is most likely functional because blood pressure increases rapidly after the start of treatment and returns to normal immediately after the discontinuation of VEGF inhibitors (van Dorst et al., 2021). Therefore, further studies to determine whether microvascular thinning is the cause or effect of the actions of VEGF inhibitors on hypertension are highly clinically important.

Animal experiments revealed that a rise in blood pressure leads to an increase in the production of reactive oxygen species in mice. Therefore, researchers have speculated that elevated blood pressure may be responsible for microvascular function and structural alterations, further contributing to the manifestation of vascular thinning (Ungvari et al., 2004; Jacobson et al., 2007). However, there is considerable evidence that microvascular changes may also be a cause rather than a consequence of hypertension. In animal models of hypertension, increased reactive oxygen species production and sparse arterioles occur even in the vasculature not exposed to hypertension (Boegehold et al., 1991). Our previous results showed that blood pressure was significantly increased in an apatinib-treated mouse model of gastric cancer, and sparsity of arteriolar vessels was detected in the mesenteric arteries of experimental model mice. Therefore, we speculate that the microcirculation damage caused by apatinib during antitumour treatment may be one of the main causes of hypertension. However, this study did not further confirm whether functional or structural vascular alterations are the cause, and it is very important to clarify this classification for the treatment of hypertension induced by TKIs. Because we did not further lower the blood pressure after the elevation of blood pressure, we investigated whether the vascular density of the superior mesenteric artery in the mice was further restored.

In addition, microvascular rarefaction is observed in the early stages of hypertension development, and microvascular rarefaction can be detected in individuals with a family history of hypertension, even if their blood pressure is normal. Similarly, in animal experiments, microvascular abnormalities occur early in the development of hypertension in SHRs (Antonios et al., 2003). In addition, the latest results from our research indicated that the administration of the ROCK inhibitor Y27632 can prevent microvascular rarefaction caused by apatinib and improve blood pressure (Wang et al., 2022a). Thus, microvascular growth disorders may have given rise to a new paradigm of hypertension treatment aimed at restoring microvascular development and branching, thereby further reducing peripheral resistance and improving the structural reduction of arterial blood pressure.

4 Relationship between microcirculation-induced hypertension and heart failure

Hypertension is an important risk factor for heart failure (Garg et al., 2021). Long-term uncontrolled hypertension can exert excessive pressure on the heart and gradually impair heart function, leading to cardiac hypertrophy and decreased myocardial contractility. Over time, this overload can impede the ability of the heart to pump blood effectively, triggering heart failure (Slivnick and Lampert, 2019;

Jackson et al., 2021; Redfield and Borlaug, 2023). The most common type of heart failure is heart failure with preserved ejection fraction (HFpEF). There are multiple mechanisms involved in the development of hypertension from HFpEF, and these mechanisms can be divided into several categories. The most common is microcirculatory dysfunction, which mainly includes an increase in inflammatory factors, the occurrence of oxidative stress, impaired endothelial function, and the occurrence of vascular endothelial fibrosis (Gryglewska et al., 2011; Paulus and Tschöpe, 2013; Stupin et al., 2021; Marra et al., 2022).

Firstly, left atrial pressure increases in hypertensive patients, with a consequent increase in left ventricular end-diastolic pressure, and left atrial remodeling occurs. This process is an indication of left ventricular diastolic dysfunction. Left atrial enlargement and dysfunction, including reduced left atrial systolic reserve, can eventually lead to the development of HFpEF (Nagai et al., 2023; Li et al., 2024). Secondly, in patients with chronic systemic inflammation such as hyperemia, systemic secreted inflammatory cytokines can cause the accumulation and inflammatory response of epicardial adipose tissue, which can promote the migration and transformation of mesenchymal stem cells and local secretion of inflammatory cytokines, leading to deep myocardial cell inflammation, increased myocardial stiffness, deep myocardial fibrosis, and finally HFpEF (Obokata et al., 2017; Gevaert et al., 2022; Gao et al., 2023; Rossi et al., 2023). In addition, microcirculation disorders also play an important role in the occurrence and development of HFpEF (Camici et al., 2020). Systemic inflammation can lead to microvascular inflammation and endothelial activation, resulting in significant structural and functional changes in cardiomyocytes and extracellular matrix, including the decrease of NO and vasodilator peptide levels, which leads to the enhancement of vasoconstriction, left ventricular stiffness and myocardial collagen-ization, and ultimately the formation of HFpEF (Vancheri et al., 2020; Agrawal et al., 2023). Overall, in most patients with hypertension, left ventricular diastolic dysfunction is the first apparent manifestation of heart failure. Our previous study (Wang et al., 2022a) showed that apatinib increased blood pressure in gastric cancer model mice, which further led to left ventricular hypertrophy and fibrosis. Therefore, exploring the mechanism through which apatinib-induced hypertension leads to HFpEF in gastric cancer mice is highly important, as is exploring drugs that can delay the progression of HFpEF.

Abnormal structure and function of miniscule arteries and capillaries results in hypoperfusion of blood flow (Mourad and Levy, 2011; Struijker-Boudier and Heijnen, 2011; Wu et al., 2023). Studies have shown that sparse microcirculation may be one of the main causes of hypertension (Ciuffetti et al., 2003). Chronic high blood pressure damages the heart and blood vessels. Pathological changes in miniscule arteries and capillaries, such as intimal thickening, fibrosis, and luminal narrowing, further inhibit blood flow perfusion (Triantafyllou et al., 2015). This microcirculatory pathology affects normal oxygen availability and nutrient delivery to the heart and other tissues (De Backer et al., 2014; Cusack et al., 2022). Ischaemia and hypoxia lead to cardiac cell damage and death, which eventually leads to weakened cardiac systolic function and the occurrence of heart failure (Premont et al., 2020). Therefore, microcirculatory impairment plays a key role in hypertension and cardiac dysfunction. Therefore, protecting microcirculation function is highly important for the prevention and treatment of hypertensive heart failure.

5 Mechanisms related to hypertension caused by TKIs

5.1 Relationship between VEGF signaling pathway and TKI-induced hypertension

The most common pathways are the vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), angiopoietin-1, and Notch signalling pathways (Viallard and Larrivée, 2017; Vimalraj, 2022; Huang et al., 2023) and so on. VEGF is one of the most important proangiogenic mediators. Recently, studies have shown that VEGF is the core factor affecting endothelial cell angiogenesis and is closely related to hypertensive microcirculation damage (Chade and Kelsen, 2010). However, the foundation of TKI antitumour therapy is the inhibition of vascular endothelial growth factor, which brings certain challenges to antitumour therapy. The latest research from our team revealed that in a mouse model of gastric cancer, the mechanism of apatinib-induced hypertension in mice may be related to the sparse vascular density of the superior mesenteric artery (Wang et al., 2022a).

VEGF signaling pathway inhibitors include monoclonal antibodies against VEGFA, vascular endothelial growth factor inhibitors (VEGF trap), monoclonal antibodies against VEGF receptors, and TKIs (Saif, 2013; Shughoury et al., 2023). TKIs are effective signaling cascade inhibitors that inhibit tumor blood vessel growth by inhibiting vascular endothelial growth factor receptor (VEGFR) (Vano et al., 2022). VEGFR-TKIs have become the main treatment for many solid malignant tumors. However, TKIs can induce vascular endothelial damage, hypertension and myocardial injury by targeting VEGFR, platelet-derived growth factor receptor (PDGFR) and stem cell factor receptor (SCFR) (van Cruijsen et al., 2009; Lennartsson and Rönnstrand, 2012). It can also damage mitochondria and affect myocardial energy metabolism through the “off-target effect", eventually leading to cardiovascular complications (Tullemans et al., 2018; Rodríguez-Agustín et al., 2023). Therefore, the incidence of cardiovascular toxicity related to VEGFR-TKIs is high, which can cause the occurrence and development of cardiovascular complications such as hypertension, left ventricular systolic dysfunction/heart failure, and atherosclerosis.

Anti-angiogenesis targeting drugs mainly act on VEGF and VEGFR. VEGFR-1, VEGFR-2 and VEGFR-3 are the major VEGF receptors (Sallinen et al., 2009; Bernatz et al., 2021). VEGF inhibitors can increase the risk of heart failure, coronary heart disease, hypertension and thromboembolic diseases through endothelial injury, vasoconstriction and remodeling, inflammatory response and platelet activation.

5.2 Relationship between RhoA/ROCK signaling pathway and TKIs-induced hypertension

In addition, the latest study from our team revealed that apatinib has a considerable therapeutic effect on a mouse model of gastric

cancer (Wang et al., 2022a). However, apatinib can also lead to an increase in blood pressure, accompanied by activation of the RhoA/ROCK signalling pathway. And the ratio between vessel thickness and lumen diameter was significantly increased in the apatinib group. We also observed that apatinib in combination with the ROCK inhibitor Y27632 did not affect the antitumour therapeutic effect of apatinib. Finally, our combined administration of ROCK inhibitors significantly reduced the increase in blood pressure in an apatinib-induced gastric cancer mouse model (Wang et al., 2022a). Therefore, Y27632 has wide application prospects for the treatment of tumour-induced hypertension.

Further studies at the cellular level have shown that knocking down the key gene LRAG in the RhoA/ROCK signalling pathway can improve the abnormal proliferation and impaired cell function of vascular smooth muscle cells caused by apatinib (Wang et al., 2022b). This is mainly because apatinib can increase blood pressure by activating the RhoA/ROCK signaling pathway. Therefore, exploring the key molecular targets of TKIs that inhibit the expression of angiogenic factors and elucidating the specific molecular mechanism involved are key for effectively promoting endothelial cell angiogenesis and achieving accurate repair of microcirculation injury in hypertension patients.

5.3 Relationship between notch signaling pathway and TKIs-induced hypertension

Notch and DLL4 are specifically expressed on vascular endothelial cells (EC). JAG has been shown to promote cell survival and proliferation, interacting with NOTCH and hematopoietic stem and progenitor cells (HSPCs) (Kangsamaksin et al., 2015). In addition, high expression of JAG promotes cancer development. We hypothesized that inhibition of Notch signaling activation would not only inhibit tumor development, but also help angiogenesis. Therefore, exploring the mechanism of angiogenesis dysfunction induced during antitumour therapy is highly clinically important.

Notch signalling pathway, as a classical signaling pathway, is closely related to cardiovascular and tumor microcirculation. Combined with our previous results, Notch signaling pathway may also be involved in TKI-induced blood pressure elevation. The regulation of the Notch signalling pathway is closely related to the proliferation, migration and tube formation of vascular endothelial cells during tumour angiogenesis. Further research on the relationship between the Notch signalling pathway and tumour-related cardiovascular disease is expected to lead to new therapeutic strategies and targets for the prevention and treatment of tumours.

5.4 Other mechanisms associated with TKIs-induced hypertension

At present, there are many studies on hypertension caused by TKIs, and the relevant mechanisms involved may be related to the following aspects: 1) decreased bioavailability of nitric oxide (Robinson et al., 2010); 2) enhanced oxidative stress (Neves et al., 2018); 3) increased secretion of endothelin-1 (Mirabito Colafella et al., 2020); 4) decreased bioavailability of prostacyclin (Wheeler-Jones et al., 1997); 5) increased bioavailability of endothelial microparticles (Neves et al., 2019); 6) sparse microvessels (Steeghs et al., 2008); 7) vascular sclerosis (Catino et al., 2018); 8) activation of renin angiotensin (Li et al., 2022); and 9) salt-sensitive hypertension (Tsai et al., 2017).

6 Role of the notch signalling pathway in the cardiovascular system

The Notch signalling pathway is a common signalling pathway that plays a certain role in cell development. The Notch protein is a transmembrane receptor that is located on the cell surface and mediates important cellular functions (Kwak et al., 2022; Takahashi et al., 2023). The interaction between the Notch protein and its ligand initiates a signalling cascade associated with cell fate that plays a key role in differentiation, proliferation, and apoptosis in many tissue types (Zhao et al., 2019; Li et al., 2020). The Notch protein, as well as its ligand, contains extracellular EGF-like repeats that interact with the DSL domain of the ligand. Activation of the Notch signalling pathway is accompanied by proteolysis, which releases the intracellular domain of Notch (NICD) (Sprinzak and Blacklow, 2021; Fang et al., 2022). The NICD is a fragment containing a RAM23 domain (RAM) that can enhance interactions with the Notch and CSL proteins, thereby facilitating the transmission of Notch to the nuclear localization signal (NLS) (Lubman et al., 2007; Johnson and Barrick, 2012). The NICD fragment can further mediate interactions with other proteins in the Notch signalling pathway (Figure 1).

The VEGF signalling pathway interacts with the Notch signalling pathway, coordinates the differentiation of arteries and veins, and is also involved in changes in vascular budding and vascular branching (Laham et al., 2003; Li et al., 2017; Souilhol et al., 2022; Li et al., 2023a). In addition, the VEGF signalling pathway can regulate key processes such as lumen remodelling of blood vessels.

Microvascular rarefaction leading to hypertension involves multiple signalling pathways, such as vascular endothelial development, angiogenesis, inflammation, oxidative stress, and endogenous short peptides (Feihl et al., 2008; Bruno et al., 2018; Do et al., 2023; Zdravkovic et al., 2023). Vascular endothelial cells play a key role in maintaining normal vascular biological function and regulating vascular tone. The abnormal regulation of vascular endothelial growth factor and platelet-derived growth factor during angiogenesis may also be involved in the occurrence of microvascular rarefaction (Cohen et al., 2023). Studies have shown that the Notch signalling pathway plays an important role in angiogenesis (Maynard et al., 2003).

The Notch signalling pathway plays a key role in the regulation of angiogenesis, especially in balancing the proangiogenic effects of the VEGF signalling pathway. The Notch signalling pathway antagonizes angiogenesis *via* VEGF signalling. It also stimulates quiescent endothelial cells and promotes apical cell formation. Thus, this pathway mediates the formation of vascular buds and the further growth of new vascular buds (Marinescu et al., 2015). Under conditions of stress, the cascade between Dll4-mediated Notch signalling and VEGFA-VEGFR2 signalling induces endothelial cells adjacent to the dominant TCS to maintain high levels of Notch signalling, thereby inhibiting their differentiation into TCShh cells. Furthermore, it can inhibit angiogenesis (Gerhardt

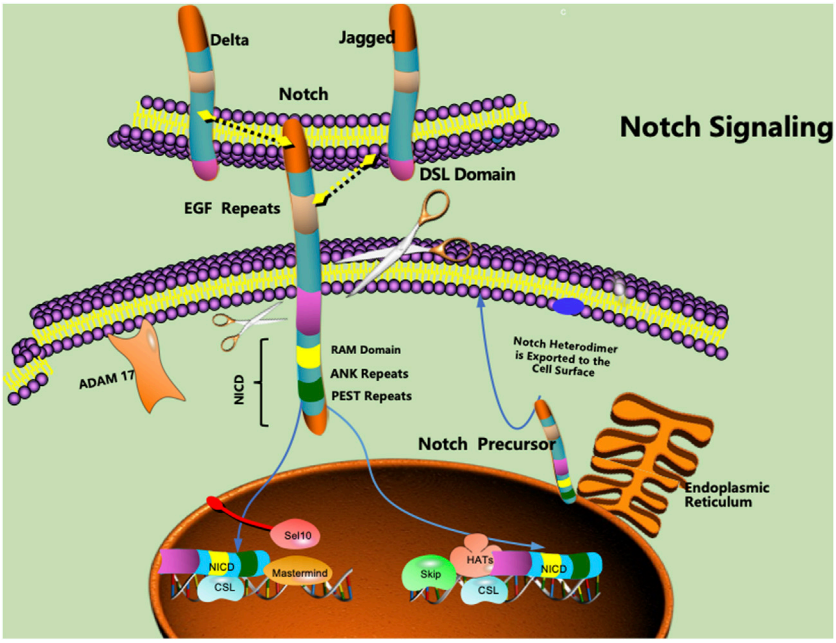

FIGURE 1
Notch proteins are cell surface transmembrane-spanning receptors that mediate critically important cellular functions through direct cell–cell contact. EGF: epidermal growth factor, NICD: intracellular domain of Notch, NLS: nuclear localization signal, CSL: CBF1/Su(H)/Lag-1.

et al., 2003; Jakobsson et al., 2010; Herbert and Stainier, 2011). Angiogenesis inhibition is thought to be controlled by Notch 1-mediated downregulation of Flt 4 and upregulation of soluble Flt 1 (sVEGFR-1/sFlt 1) (Chappell et al., 2009; Trindade et al., 2012). In particular, upregulation of sFlt1 reduces local VEGF bioavailability and inhibits angiogenesis. The Notch ligand Jag 1 is expressed mainly in stem cells and is thought to specifically block Dll 4-Notch 1 signalling in apical cells (Benedito et al., 2009). In the cardiovascular system, excessive activation of the Notch signalling pathway can lead to vascular endothelial cell proliferation and congestion and an increase in vessel wall thickness, which leads to vascular stenosis and hypertension (Niessen and Karsan, 2007; MacGrogan et al., 2018; Gomez et al., 2021).

The Notch signalling pathway is also involved in the occurrence and development of heart failure. Activation of Notch signalling can lead to cardiomyocyte proliferation and hypertrophy and can also affect angiogenesis and repair processes in the cardiovascular system (Fortini et al., 2014; Matsushita et al., 2023). These changes may adversely affect myocardial function and lead to the development of heart failure. Notch1 is a transmembrane receptor found in a variety of cells, including smooth muscle cells and endothelial cells in the cardiovascular system. During heart wall formation, Notch signalling regulates the ratio of cardiomyocytes to noncardiomyocytes by inhibiting myogenesis, thereby further promoting atrioventricular canal remodelling and maturation and heart valve formation (Niessen et al., 2011; Peng et al., 2023). Activation of the Notch1 signalling pathway is achieved mainly through the binding of the Notch1 receptor to its ligand. When the Notch1 receptor binds to its ligand, secondary cleavage occurs, and the active region of the Notch1 receptor is released. Then the receptor enters the nucleus and interacts with transcription factors to promote gene transcription and expression (Wang S. et al., 2022). Studies have also shown that activation of the Notch1 signalling pathway is closely related to cardiac function and can affect the proliferation and differentiation of cardiomyocytes, thus affecting the development and function of the heart (Pahlavani, 2022). In addition, activation of the Notch1 signalling pathway can also regulate the expression of the actin gene in cardiac cells, affecting cardiac contractility and cardiac contraction rhythm (Hrstka et al., 2017). Therefore, the Notch signalling pathway plays an integral and critical role in maintaining and regulating blood pressure and cardiac function.

7 Role of the notch1 signalling pathway in tumour systems

The Notch signalling pathway is involved in cell-to-cell interactions and communication and plays an important role in embryonic development and the maintenance of tissue homeostasis in adult organisms. Moreover, aberrant Notch signalling pathway activity has also been found to be closely related to the occurrence and development of a variety of tumour types.

In terms of tumours, studies have shown that abnormal activation of the Notch1 signalling pathway is related to the occurrence and development of a variety of diseases, such as tumour proliferation and metastasis and abnormal responses of the immune system (Yang et al., 2018; Peng et al., 2023). Therefore, we hypothesized that inhibition of the Notch1 signalling pathway may ameliorate the adverse effects on blood pressure and cardiac function. Therefore, what is the underlying mechanism involved in reducing blood pressure and cardiac dysfunction? This will be another important dimension that needs to be studied.

In hepatocellular carcinoma (HCC), Notch signalling is activated by different ligands and plays a polymorphic role depending on the cell type, affecting tumour growth, invasive ability, and stem cell-like properties (Giovannini et al., 2021). Thus, interfering with Notch signalling may be a promising therapeutic approach. In tumour therapy, the downregulation of Notch1 can achieve synergistic effects and reduce chemoresistance when targeted drugs are used alone or in combination with chemotherapy. In addition, Notch mutations have been proposed to be predictive biomarkers for immune checkpoint blockade therapy in many cancers (Zhou et al., 2022; Li et al., 2023b).

8 Possible treatment of hypertension and myocardial damage caused by the notch signalling pathway

Recently, it has been observed that Epac1 can negatively regulate the Notch signalling pathway. Epac1 knockdown in endothelial cell lines resulted in a significant increase in the intracellular Notch1 protein level, and Epac1 knockdown increased the protein levels of NICD and DLL4, thereby further inhibiting angiogenesis. Therefore, overexpression of Epac1 may be an effective way to alleviate microvascular rarefaction (Fritz et al., 2015; Lan et al., 2020; Slika et al., 2023). Interestingly, studies have shown that Epac1 knockdown can inhibit pathological angiogenesis but has no significant effect on physiological angiogenesis, which is worthy of attention and discussion (Lan et al., 2020). Previous studies have shown that Epac1 is a β-secretase enzyme that inhibits the activation of the Notch signalling pathway (Fujita et al., 2017; Tan et al., 2022). In addition, Epac1 has been shown to enhance the VEGF signalling pathway and promote pathological angiogenesis (Namkoong et al., 2009; Ramos et al., 2018). Therefore, we speculate that the occurrence of hypertension caused by TKIs may lead to microcirculation disorders and microvascular thinning, and overexpressing the Epac1 gene may be a very meaningful way to improve or treat microvascular thinning caused by TKIs. NOTCH mutations can be used as predictive markers for treatment with immune checkpoint blockade in some tumors. In summary, it is necessary to comprehensively evaluate the NOTCH pathway from a new perspective, so as to apply it in the subsequent clinical diagnosis and treatment.

9 Mitochondrial dysfunction may also be involved in the regulation of notch signalling

In addition, Epac1 can play a role in cell-to-cell molecular conduction by increasing its interaction with macromolecular complexes, including voltage-dependent anion channel 1 (VDAC1), chaperone glucose-regulated protein 75 (GRP75), and inositol triphosphate receptor 1 (IP3R1). The interaction between Epac1 and macromolecular complexes can further promote the exchange of Ca2+ between the endoplasmic reticulum and mitochondria, which can eventually lead to mitochondrial Ca2+ overload and the opening of mitochondrial permeability transition pores (Fazal et al., 2017). Therefore, Epac1 is expected to be a new target for the treatment of ischaemic myocardial injury.

Mitochondria are important organelles for cellular energy production, and dysfunction of these organelles plays a key role in the emergence and progression of cardiovascular diseases (Poznyak et al., 2021; Wu et al., 2022). Normally, mitochondrial proteostasis is monitored by an extensive system: the mitochondrial unfolded protein response (UPRmt) is activated when mitochondria are stimulated by misfolded protein stress. The latter promotes the upregulation of ClpP, HSP6, HSP-60, ATFS-1 and other markers through a series of signalling cascades to alleviate mitochondrial stress (Merkwirth et al., 2016; Kumar et al., 2022; Xin et al., 2022; Guo et al., 2023). Under conditions of stress, cells can protect mitochondria by activating the UPRmt. It has been shown that the inhibition of Epac1 prevents ferroptosis-induced cell death and disruption of mitochondrial integrity, whereas the inhibition of Epac2 has a limited effect (Laudette et al., 2021; Musheshe et al., 2022). However, the potential effects of Epac on mitochondrial function are still unclear, and additional experiments are needed for further elucidation.

Several studies have shown that UPRmt activation is accompanied by the upregulation of the mitophagy markers PINK1, PARK2, BNIP3, P62, and LC3 and the mitochondrial oxidative phosphorylation (OXPHOS) markers Cox5a, Cox2, Nd1, and Sdhc. These results indicate that the UPRmt, mitophagy and OXPHOS play synergistic roles in maintaining mitochondrial protein balance and mitochondrial function (Kang et al., 2017; Martinez et al., 2017; Sorrentino et al., 2017; Poole and Macleod, 2021). The latest article published in NATURE refers to a series of mitochondrial stress responses that cause abnormal changes in the UPRmt, mitophagy, and OXPHOS under specific pathological or stimulus conditions, such as the mitochondrial stress response (MSR) (Ruan et al., 2017; Sorrentino et al., 2017; Jakobsen et al., 2019). The MSR is a key mechanism regulating mitochondrial protein balance and the mitochondrial stress response. EPAC1 can activate the UPRmt and protect mitochondrial function. Additionally, it is a key molecule that regulates the UPRmt and mitophagy (Gariani et al., 2016; Jayarajan et al., 2019; Aslam and Ladilov, 2021).

10 Summary

TKIs can induce vascular endothelial damage, hypertension and myocardial injury by acting on the targets VEGFR, platelet-derived growth factor receptor (PDGFR) and stem cell factor receptor (SCFR). It can also damage mitochondria and affect myocardial energy metabolism through “off-target effects", eventually leading to cardiovascular complications. Therefore, the goal of antitumour therapy is to maximize the antitumour effect while reducing treatment-related cardiovascular events. Therefore, studying the mechanism of hypertension during the treatment of cancer patients with TKIs and finding compounds or key factors that can reduce blood pressure without affecting antitumour efficacy are highly valuable. We hypothesize that inhibition of Notch signalling could ameliorate TKI-induced microvascular rarefaction, thereby further ameliorating the increase in blood pressure or the resulting cardiac dysfunction caused by microvascular rarefaction. Overexpression of Epac 1, a key gene in the Notch1 signalling pathway, can improve the pathological

vascular inhibition induced by TKIs. The mechanism by which apatinib, a representative TKI, induces microcirculation damage, hypertension and heart failure through the Notch1 signalling pathway will also be explored at the cellular and animal levels to provide clinical guidance for patients with hypertension induced by cancer treated with TKIs.

In summary, side effects such as cardiovascular toxicity caused by antitumour therapy with TKIs have become one of the main factors limiting antitumour therapy with TKIs. For the treatment of such hypertension, the effect of traditional antihypertensive drugs is not ideal, and traditional antihypertensive drugs are also closely related to the occurrence and development of some tumours. Exploring the underlying mechanism of cardiovascular complications caused by antitumour treatment with TKIs is crucial for ensuring the smooth clinical application of TKIs, and identifying this mechanism is also an urgent need. New directions for improving the treatment of hypertension induced by these drugs should be explored. The ultimate goal of our team will be to improve the clinical outcome as soon as possible and to provide greater benefits for cancer patients.

Author contributions

WW: Methodology, Resources, Writing–original draft, Writing–review and editing. GL: Investigation, Writing–review and editing. JM: Writing–review and editing. XF: Formal Analysis, Writing–review and editing. JL: Conceptualization, Writing–review and editing. QS: Supervision, Writing–review and editing. JY: Data curation, Writing–review and editing. QH: Conceptualization, Investigation, Supervision, Validation, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Yusof Kamisah,
Faculty of Medicine Universiti Kebangaan Malaysia, Malaysia

REVIEWED BY
Songqi Tang,
Hainan Medical University, China
Ahmad Khusairi Azemi,
Universiti Malaysia Terengganu, Malaysia

*CORRESPONDENCE
Rong-Sheng Tong,
tongrs@126.com
Cheng Peng,
pengchengchengdu@126.com

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Shenfu injection: a review of pharmacological effects on cardiovascular diseases

Fei-Fei Xu[1,2], Xiao-Fang Xie[1], Hai-Yan Hu[3], Rong-Sheng Tong[2]* and Cheng Peng[1]*

[1]State Key Laboratory of Southwestern Chinese Medicine Resources, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]Department of Pharmacy, Personalized Drug Therapy Key Laboratory of Sichuan Province, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China, [3]Sichuan Nursing Vocational College, Chengdu, China

Shenfu injection (SFI), composed of ginseng and aconite, is a Chinese patent developed from the classic traditional prescription Shenfu Decoction created more than 700 years ago. SFI has been widely used in China for over 30 years for treating cardiovascular diseases. The main components in it include ginsenosides and aconitum alkaloids. In recent years, the role of SFI in the treatment of cardiovascular diseases has attracted much attention. The pharmacological effects and therapeutic applications of SFI in cardiovascular diseases are summarized here, highlighting pharmacological features and potential mechanisms developments, confirming that SFI can play a role in multiple ways and is a promising drug for treating cardiovascular diseases.

KEYWORDS

Shenfu injection, cardiovascular disease, heart failure, pharmacology, TCM (traditional Chinese medicine), clinical trial

1 Introduction

Cardiovascular diseases (CVDs) remain the predominant cause of mortality and morbidity worldwide over the past 20 years, including atherosclerosis, coronary heart disease, arrhythmia, hypertension, cardiomyopathy, stroke and heart failure (Parikh et al., 2018; Benjamin et al., 2019; Feng et al., 2019; Makhmudova et al., 2021). According to the World Health Organization (WHO) Report 2021, noncommunicable diseases (NCDs) kill more than 40 million people every year, and CVDs are the world's leading cause of death, accounting for almost one in three of all reported deaths globally. Data from the World Heart Report 2023 shows that 20.5 million people died from CVDs in 2021 (Mariappan et al., 2023). CVDs are caused by a variety of pathological factors, such as atherosclerosis, hypertension, hyperlipidemia, diabetes mellitus and so on, associated with energy metabolism disorder, mitochondrial structure abnormality, oxidative stress injury, cardiomyocyte apoptosis, inflammatory reaction, but the specific pathogenesis has not yet been fully elucidated (Parikh et al., 2018; Benjamin et al., 2019; Feng et al., 2019; Makhmudova et al., 2021). Based on the complex pathophysiologcial mechanisms, there are numerous drugs recommended for the treatment of CVDs, including angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, β-receptor antagonists, vasodilators, diuretics, α-receptor antagonists, positive inotropes, lipid-lowering drugs, antiarrhythmics, calcium channel blockers, *etc.* However, their potential serious adverse effects caused by these drug, such as hyperkalemia, cardiac depression, and electrolyte disturbance, cannot be ignored

FIGURE 1
Shenfu injection.

FIGURE 2
Ginseng Radix et Rhizoma Rubra.

FIGURE 3
Aconiti lateralis radix praeparata.

(Alhawassi et al., 2018; Núñez-Acevedo et al., 2018; Pall et al., 2021). Therefore, folk medicine is widely used to treat CVDs, among which traditional Chinese medicine (TCM) is well known in the world. Along with the long history of development for TCM, some classic recipes for the treatment of CVDs have been used in the clinic since then. Zhigancao Decoction, originated from *Treatise on Febrile Diseases* in the Eastern Han Dynasty (25–280 AD), is composed of Glycyrrhiza uralensis Fisch [Leguminosae; Glycyrrhizae Radix et Rhizoma], Zingiber officinale Rosc [Zingiberaceae; Zingiberis Rhizoma Recens], Cinnamomum cassia Presl [Lauraceae; Cinnamomi Ramulus], Panax ginseng C.A.Mey [Araliaceae; Ginseng Radix et Rhizoma Rubra], Rehmannia glutinosa Libosch [Scrpophulariaceae; Rehmanniae Radix], *Equus asinus* L [Equidae; Asini Corii Colla], Ophiopogon japonicus (L.f) Ker-Gawl [Liliaceae; Ophiopogonis Radix], Cannabis sativa L [Moraceae; Cannabis Fructus], Ziziphus jujuba Mill [Rhamnaceae; Jujubae Fructus], and used to treat arrhythmia and heart failure (Xiong, 2019; Zhang N. et al., 2021; Wu et al., 2021; Yang Y. et al., 2022). Xuefu Zhuyu Decoction, recorded in the classic *Yi Lin Gai Cuo* in the Qing dynasty (1830 AD), composed of eleven commonly used herbs, including Prunus persica (L.) Batsch [Rosaceae; Persicae Semen], Carthamus tinctorius L [Compositae; Carthami Flos], Angelica sinensis (Oliv.) Diels [Umbelliferae; Angelicae Sinensis Radix], Rehmannia glutinosa Libosch [Scrpophulariaceae; Rehmanniae Radix], Achyranthes bidentata Bl [Amaranthaceae; Achyranthis Bidentatae Radix], Ligusticum chuanxiong Hort [Umbelliferae; Chuanxiong Rhizoma], Platycodon grandiflorum (Jacq.) A. DC [Campanulaceae; Platycodonis Radix], Paeonia lactiflora Pall [Ranunculaceae; Paeoniae Radix Rubra], Citrus aurantium L [Rutaceae; Aurantii Fructus], Glycyrrhiza uralensis Fisch [Leguminosae; Glycyrrhizae Radix et Rhizoma], Bupleurum chinense DC [Umbelliferae; Bupleuri Radix], is used to treat hyperlipidemia and coronary heart disease (Wang and Qiu, 2019; Zhang S. et al., 2021; Yang et al., 2023). Zhenwu Decoction, was firstly recorded in *Treatise on Febrile Diseases*. It inculdes five herbs: Poria cocos (Schw.) Wolf [Polyporaceae; Poria], Paeonia lactiflora Pall [Ranunculaceae; Paeoniae Radix Alba], Zingiber officinale Rosc [Zingiberaceae; Zingiberis Rhizoma Recens], Aconitum carmichaelii Debx [Ranunculaceae; Aconiti Lateralis Radix Praeparata], Atractylodes macrocephala Koidz [Compositae; Atractylodis Macrocephalae Rhizoma], which is applied to treat chronic heart failure (Tang et al., 2018; Han et al., 2022). It is believed in TCM that CVDs is related to the imbalance of Qi, Xue, Yin and Yang in the human body. When Qi and Yang is insufficient, CVDs are prone to occur.

SFI (Figure 1) is widely used in China to treat numerous ailments, including shock (Zhang X. et al., 2020; Wang et al., 2022; Zhang and Li, 2023), pulmonary fibrosis (Liu et al., 2021), sepsis (Luo et al., 2021; Li X. et al., 2022; Xu et al., 2022), pneumonia (Niu et al., 2021; Shi et al., 2022), cancer (Gao and Zhang, 2023; Wen et al., 2023), cercerebral infarction (Zhou et al., 2020), CVDs, and has shown promising results. With development of pharmacological research, SFI has been identified as an effective drug for the treatment of CVDs.This paper reviews the latest reports in the past 20 years (2003–2022) from PubMed, Web of Science, and National Knowledge Infrastructure (CNKI) using the keywords “Shenfu injection” and “cardiovascular diseases”. The pharmacological action and therapeutic application of SFI in treating CVDs were discussed, and its pharmacological characteristics and potential mechanism was emphasized.

2 SFI -basic characteristics and history of use

SFI is a commonly used traditional Chinese medicine injection that has been used in clinical for over 30 years (Liu et al., 2021). It originated from the traditional Chinese classical formula "Shenfu Decoction", which was first recorded in *Yan's Prescriptions for Rescuing Lives* in the Song Dynasty (1253 AD). SFI is composed of Panax ginseng C.A.Mey [Araliaceae; Ginseng radix et rhizoma rubra] (RG) (Figure 2) and Aconitum carmichaelii Debx [Ranunculaceae; Aconiti lateralis radix praeparata] (RA) (Figure 3), which has the function of restoring Yang and invigorating Qi (Pei et al., 2021; Zhou et al., 2022). The existing studies reported that RG can be used to treat coronary heart disease and atherosclerosis by reducing blood lipid levels and improving inflammation (Hernández-García et al., 2019; Lu et al., 2019; Im, 2020). Additionally, it can inhibit arrhythmia by affecting the ion channels, such as activating potassium channel while blocking calcium channel and sodium current (Liu Z. et al., 2019; Gou et al., 2020). Furthermore, by ameliorating mitochondrial function and reducing oxidative damage in cardiomyocytes, it can prevent ventricular remodeling and heart failure. Moreover, it has the potential to improve the function of vascular endothelial cells, thereby lowering blood pressure (Yang F. et al., 2022; Liu et al., 2022). Meanwhile, RA has cardiotonic effects by accelerating β-adrenergic receptor synthesis (Tong et al., 2021), has anti-inflammatory effects through the Toll-like receptor4/Nuclear factor κB (TLR4/NF-κB) pathway (Yan et al., 2020), and has anti-arrhythmic effects (Wang et al., 2023). SFI, composed of RA and RG, is a common drug for the treatment of CVDs.

Modern chemical studies have shown that SFI mainly contains ginsenosides, aconite alkaloids, organic acids, nucleosides, amino acids and other components (Song et al., 2015). Ginsenosides and aconite alkaloids are the main active components of SFI. The content of ginsenosides is 676–742 μg/mL, and the content of aconite alkaloids is 3–7 μg/mL (Yang et al., 2014; Ge et al., 2015; Song et al., 2015). It is known that aconite has certain toxicity, and the use of RG and RA in combination can achieve the effect of potentiation and detoxification. Ginsenosides can promote the metabolism of the toxic component aconitine, prolong the elimination half-life of active ingredients such as hypaconitine, benzoylmesaconine and songorine, and significantly increase the *in vivo* exposure of active ingredients. At the same time, some studies have found that ginseng can inhibit the ion disorders, toxicity in calcineurin-nuclear factor of activated T cells (CaN-NFAT3) pathway and inhibition of the cytochrome P450 2J3 (CYP2J3) expression caused by aconitine, and enhance the antioxidant effect of myocardial cells (Liu et al., 2020; Yang et al., 2021; Chen Z. Y. et al., 2022; Bao et al., 2023). Therefore, the compatibility of aconite and ginseng has the effect of 'reducing toxicity and increasing efficiency'.

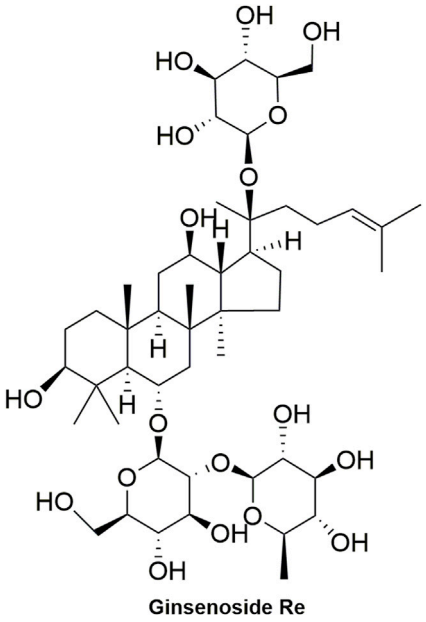


FIGURE 4
Structural formula of ginsenoside Re.

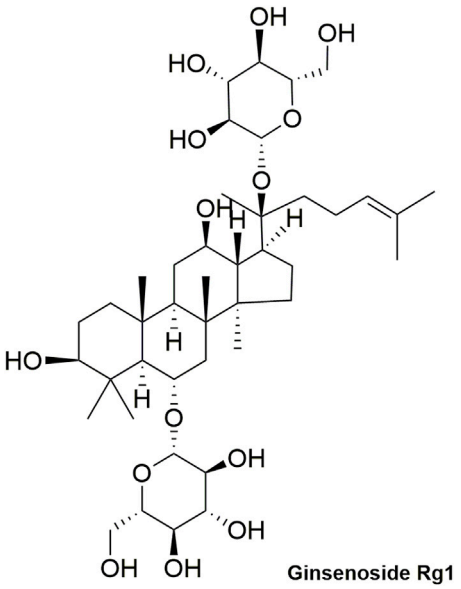


FIGURE 5
Structural formula of ginsenoside Rg1.

3 Bioavailability and metabolism of SFI

Pharmacokinetic data of rodents show that aconitum alkaloids can be rapidly eliminated after intravenous injection of SFI. Protopanaxatriol (PPT) ginsenosides such as ginsenoside Re (Figure 4), Rg1 (Figure 5) and Rg2 (Figure 6) can be rapidly excreted into bile when ginsenosides was given to rats (Cai et al., 2022). The elimination rate of protopanaxadiol ginsenosides such as ginsenoside Rb1 (Figure 7), Rd (Figure 8) and Rh2 (Figure 9) is slower than that of PPT ginsenosides (Li et al., 2015; Zhang et al., 2016; Shen et al., 2021). The pharmacokinetic properties of ginsenosides (ginsenoside Rg1, ginsenoside Rb1, ginsenoside Rc (Figure 10)) and aconitine alkaloids (benzoylmesaconine (Figure 11), aconitine (Figure 12)) in SFI showed a linear relationship in the dose range of 2–8 mL/kg (Zhang et al., 2016; Li S. et al., 2022).

Modern studies have shown that SFI is almost safe at conventional therapeutic doses, and the incidence of adverse reactions is relatively low

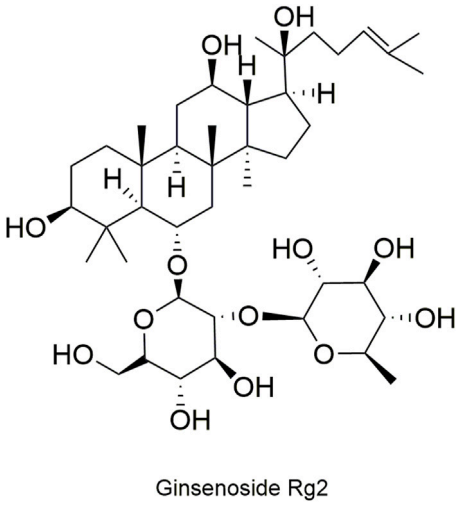


FIGURE 6
Structural formula of ginsenoside Rg2.

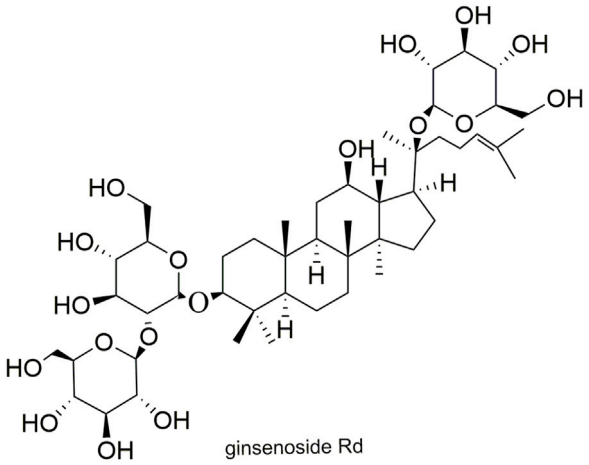


FIGURE 8
Structural formula of ginsenoside Rd.

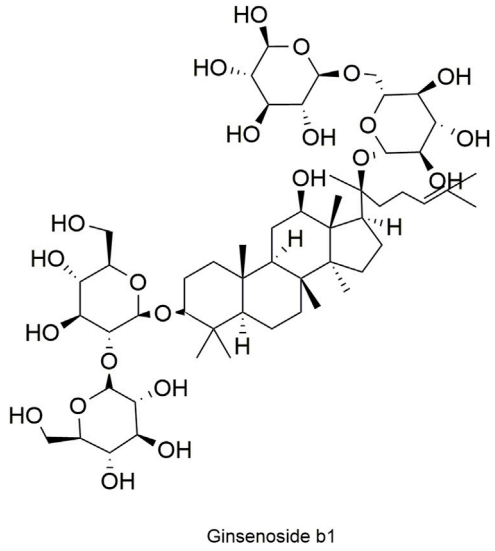


FIGURE 7
Structural formula of ginsenoside Rb1.

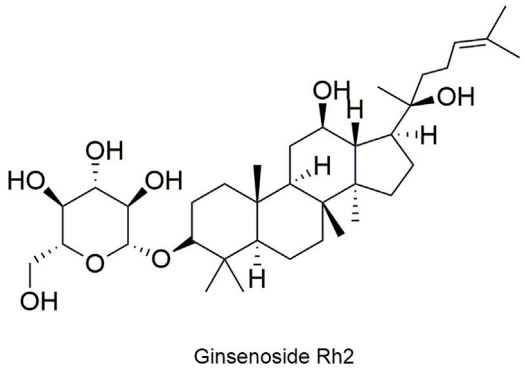


FIGURE 9
Structural formula of ginsenoside Rh2.

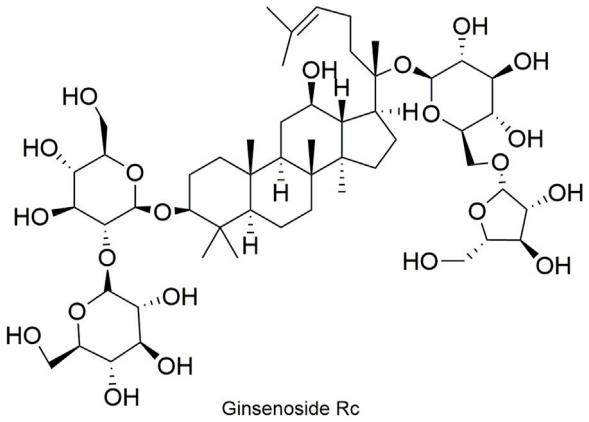
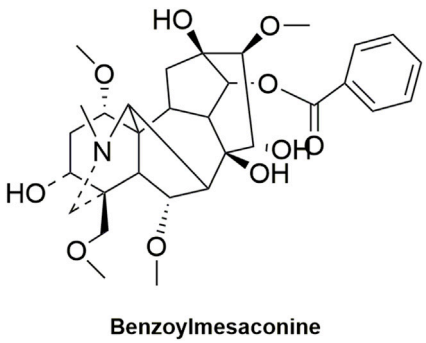


FIGURE 10
Structural formula of ginsenoside Rc.

(0.076%), such as rash, itching, nausea, vomiting, dizziness, abdominal pain, and palpitation (Wang Z. F. et al., 2017).

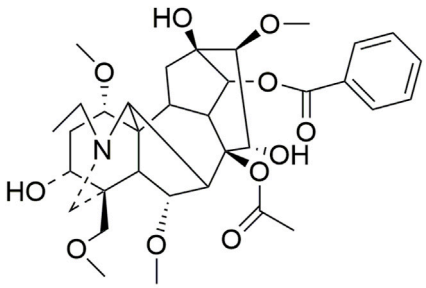
4 Pharmacological activities of SFI on CVDs

Many studies have confirmed that SFI has therapeutic effects on a variety of CVDs, such as myocardial hypertrophy, heart failure, ischemia-reperfusion injury, cardiac arrest, and arrhythmia. Its mechanism of action is mainly related to reducing inflammation



Benzoylmesaconine

FIGURE 11
Structural formula of benzoylmesaconine.



Aconitine

FIGURE 12
Structural formula of aconitine.

through NF-κB signaling pathway, oxidative stress by reducing free radical damage, dilating blood vessels by increasing nitric oxide (NO) content, decreasing fibrosis through TGF-?/Smads signaling pathway and reducing apoptosis by increasing the expression of apoptosis proteins (Figure 13).

4.1 Cardiac hypertrophy and heart failure

Cardiac hypertrophy is mainly manifested as thickening of ventricular walls and an increase in cardiomyocyte size, closely related to cardiac fibrosis and heart failure (Feng et al., 2019). As time progresses and in settings of sustained stress, cardiac hypertrophy and fibrosis will eventually lead to heart failure (Gallo et al., 2019; Zhao D. et al., 2021; Methatham et al., 2021). Inhibiting cardiac hypertrophy and fibrosis is an effective way to treat heart failure.

TGF-?/Smads plays a key role in the pathogenesis of myocardial fibrosis. The previous research suggested that TGF-?1 bind to receptor, recruited and phosphorylated type I receptor, induced phosphorylation of Smad2 and Smad3. The phosphorylated Smad2 and Smad3 formed a trimer complex with Smad4. Then the complex transferred into the nucleus and regulated the transcription of target genes, regulating the synthesis of collagen fibers and the activation of fibroblasts. Smad7 can competitively bind to the type I receptor of TGF-?1 and inhibit the signal transduction of TGF-?1/Smads pathway (Stewart et al., 2018; Wang L. et al., 2021). Ni et al. (2017) found that SFI can effectively improve cardiac function in the rate model of congestive heart failure (CHF) and attenuate ventricular remodeling and myocardial fibrosis by regulating TGF-?/Smads signaling pathway, upregulating Smad7 and downregulating TGF-?1, Smad2 and Smad3 gene expression.

Inflammatory response is trigged in the myocardial infarction area which can result in cardiac remodeling and heart failure, accumulating high levels of monocytes and neutrophils (Halade and Lee, 2022). Inflammatory cells can also stimulate repair pathways, with increasing the content of extracellular matrix in the myocardium, including matrix metalloproteinase-9 (MMP-9) and collagen (Valiente-Alandi et al., 2018). SFI has anti-inflammatory effect, which can reduce the content of inflammatory factors such as tumor necrosis factor-α (TNF-α), interleukin-1? (IL-1?) and interleukin-6 (IL-6) in serum of rats, and also reduce the expression of fibronectin, collagen I, collagen III and MMP-9 protein (Ni et al., 2017; Guo et al., 2022).

Endothelial function is essential for maintaining normal vasomotor function, and disruption of this function can result in vasomotor dysfunction (Sabe et al., 2022). When heart failure occurs, endothelial function is impaired, with vasoconstrictor substances increasing, and vasodilator substances decreasing (Monteiro et al., 2019). NO is the most famous vasodilator, while endothelin-1 (ET-1) is the most widely recognized vasoconstrictor (Miyauchi and Sakai, 2019). The production of NO mainly depends on the activity and quantity of endothelial nitric oxide synthase (eNOS). When the expression of eNOS mRNA increases, the number of eNOS synthesis increases. When eNOS binds with calmodulin (CaM), the activity of eNOS enchance, adversely, when binds with caveolin-1 (Cav-1), decrease. The phosphorylation of eNOS depends on the phosphatidylinositol 3-kinase/protein kinase B (PI3K-Akt) signaling pathway, which produces phosphorylated tyrosine residues, thereby provides an anchor site for the recruitment of PI3K to the membrane (Garcia and Sessa, 2019; Suvorava et al., 2022). Zhu et al. (2020) found that SFI can increase the expression of eNOS mRNA and CaM and promote NO synthesis, decrease the expression of Cav-1 and ET-1 content, promote eNOS phosphorylation via the PI3K/Akt signaling pathway.

MicroRNAs (miRNAs), encoded by myosin heavy chain (MHC) genes, are important regulatory factors of CVDs (Omidkhoda et al., 2019). MiR19a-3p plays an important role in cardiac hypertrophy. Myocytes specific enhancer factor 2A (MEF2A) is a target gene of miR-19a-3p and is highly expressed in cardiac hypertrophy while miR-19a-3p has low expression (Mao et al., 2018). SFI can upregulate the expression of miR-19a-3p, and downregulate the expression of MEF2A and ?-myosin heavy chain (?-MHC), so attenuate cardiac hypertrophy (Mao et al., 2018).

4.2 Ischemia-reperfusion injury

Myocardial ischemia-reperfusion injury is a significant factor that has a negative impact on the prognosis of myocardial infarction patients, causing myocardial stunning, no-reflow phenomena, reperfusion arrhythmia, and even permanent cardiomyocyte death. Therefore, it is critical to understand the mechanism of myocardial ischemia reperfusion and develop efficient treatments (Mokhtari-Zaer et al., 2018; Deng, 2021).

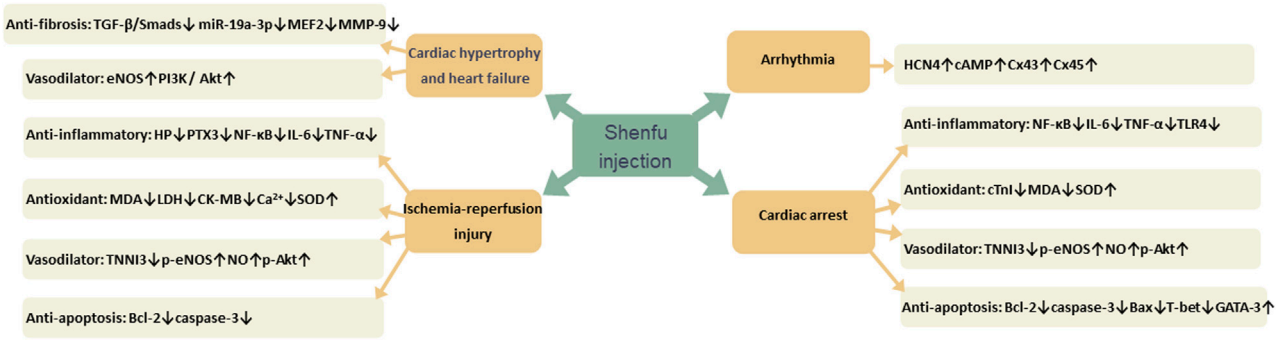


FIGURE 13
The pharmacological effect of Shenfu injection (SFI) on cardiovascular diseases.

Apoptosis is involved in the pathogenesis of various CVDs and plays an important role in myocardial ischemia-reperfusion injury. Bcl-2 family proteins are important regulators of the process, prevent apoptosis by acting upstream of apoptosis proteins, such as caspase-3 and caspase-9 (King et al., 2023; Sahoo et al., 2023). Previous research found that SFI can upregulate the anti-apoptosis protein Bcl-2 and inhibit the consecutive activation of caspase-3 and caspase-9, both of which are intimately associated to apoptosis (Cao et al., 2005; Wang et al., 2009; Guo et al., 2016).

Oxidative stress is a risk factor for CVDs, and abnormally increased reactive oxygen species (ROS) is the main cause of oxidative stress. ROS combined with proteins and lipids damage cardiomyocytes (Peng et al., 2022). The superoxide dismutase (SOD) is the major antioxidant enzyme that degrade superoxide (Eleutherio et al., 2021). The glutathione system is widely recognized as one of the most potent endogenous antioxidant systems within cardiovascular system. Glutathione, one of the endogenous antioxidant molecules, can directly scavenge ROS caused by myocardial ischemia (Panday et al., 2020; Tan et al., 2023). Taurine is a common endogenous sulfur-containing amino acid with antioxidant activity and can inhibit the abnormal increase of ROS (Li et al., 2020). SFI dramatically decreased glutathione and taurine, increased SOD activity, and inhibited the rise in malondialdehyde (MDA), which is closely related to oxidative stress (Zheng et al., 2004; Cao et al., 2005; Wu et al., 2019).

Numerous studies have revealed that NO is a vasodilator with the ability to operate on cardiomyocytes and vascular endothelium via a variety of signaling pathways (Boycott et al., 2020; Cyr et al., 2020). The action of eNOS is primarily responsible for NO generation. NO generated by eNOS phosphorylation induces soluble guanylate cyclase (sGC) to create cyclic guanosine monophosphate (cGMP), a second messenger with cardiovascular protective properties (Mount et al., 2007; Zhang Q. et al., 2020; Lee et al., 2021). SFI activated eNOS phosphorylation via Akt, thereby promoting the production of NO (Wu et al., 2011; Wang et al., 2018).

4.3 Cardiac arrest

Cardiac arrest (CA), one of the leading causes of death, has a significant impact on the public health, particularly due to its persistent increase worldwide (Vazquez and Sudhir, 2023). Post-cardiac arrest syndrome (PCAS) is a group of diseases characterized by systemic ischemia/reperfusion injury, hypoxic brain injury and myocardial dysfunction after cardiac arrest (Jou et al., 2020). It is associated with cardiovascular ischemia/reperfusion injury and cardiovascular toxicity, including factors such as excessive activation of inflammatory cytokines and catecholamines (Lazzarin et al., 2022). Matrix metalloproteinases, tumor necrosis factor, and interleukins each have a special prognostic function in PCAS. High inflammatory cytokine levels have been linked to poor neurologic and/or death outcomes (Jou et al., 2020).

NF-κB signaling pathway is one of the important pathways regulating inflammation and plays a key regulatory role in the occurrence and development of various CVDs (Cheng et al., 2023). Study have reported that in cardiac arrest swine, SFIremarkedly decreased levels of many inflammatory cytokines, such as TNF-α, IL-6, mRNA and protein levels of myocardial TLR4 and NF-κB (Gu et al., 2021). TLR4, as a 'portal' protein, regulates the initiation of the inflammatory chain reaction of the body's immunity and mediates the inflammatory response (Fitzgerald and Kagan, 2020).

Na^+-K^+-ATPase enzyme and Ca^{2+}-ATPase enzyme, ubiquitous enzymes in the heart, play a crucial role in process of CVDs (Fedosova et al., 2021). Na^+-K^+-ATPase transports two Na^+ ions extrude out of the cell in exchange for one K^+ ions, thereby maintaining the concentration gradients across the cell membrane (Fedosova et al., 2021; Obradovic et al., 2023). Ca^{2+}-ATPase, a crucial role for cellular Ca^{2+} homeostasis, maintains normal intracellular calcium concentration and prevents calcium overload (Nguyen et al., 2023; Ye et al., 2023). It is reported that SFI increased Na^+-K^-ATPase and Ca^{2+}-ATPase activity (Ji et al., 2011).

4.4 Arrhythmia

The normal electrical activity of the heart is initiated by special pacemaker cells located in the sinoatrial node (Liang et al., 2021). Dysfunction or loss of pacemaker cells can cause arrhythmia (Liu and Yuan, 2021b). The transplantation of stem cells is regarded as a kind of feasible treatment for arrhythmia (Sattayaprasert et al., 2020). It has been reported that bone marrow mesenchymal stem cells (BMSCs) with specific phenotypes can be transformed into

TABLE 1 Clinical trial of SFI in cardiovascular disease.

Disease	Number of patients	Dose of SFI (mL)	Duration	Route of administration	Outcome measures	References
acute heart failure	80 patients	50	7 days	i.v	cardiac function, clinical symptoms and quality of life	Wang et al. (2019b)
	55 patients	100	24 h	i.v	CI, cardiac output and stroke volume index	Li et al. (2022a)
Chronic heart failure	80 patients	50	7 days	i.v	LVEF, LVED, BNP,Fas, TNF-α, IL-6, mortality, readmission rate	Liu et al. (2015)
	171 patients	60	2 weeks	i.v	the all-cause mortality,6MWT	Wang et al. (2017a)
	55 patients	50	7 days	i.v	Cardiacfunction, LVEF, NT-proBNP, TNF-α, IL-6	Gao et al. (2021)
	91 patients	40	7 days	i.v	Cardiac function, LVEF, NT-proBNP, BNP	Guo et al. (2021)
coronary syndrome	74 patients	40	4–6 h	i.v	the level of NGAL in urine	Guo et al. (2017)
myocardial infarction	20 patients	80	5 days	i.v	the area of myocardial infarction	Wang et al. (2021b)
cardiac arrest	492 patients	100	28 days	i.v	28-day and 90-day survival rates, the mechanical ventilation time and hospitalization time	Zhang et al. (2017)

pacemaker-like cells after special treatment (Chauveau et al., 2014). Moreover, HMSCs possess the capability to regulate arrhythmia substrates by altering their secretory groups in diseases (Sattayaprasert et al., 2020).

In vitro, SFI can activate inward pacemaker current of BMSCs in a concentration-dependent manner, increase HCN4 expression and cAMP content in BMSCs, induce BMSCs proliferation, promote their differentiation into pacemaker-like cells (Zhao X. et al., 2021). The HCN4 gene serves as the molecular basis for the pacemaker current, contributing significantly to inward current during depolarization and playing a crucial role in the generation and autonomous regulation of heart rate (Bucchi et al., 2012; D'Souza et al., 2021; Hoekstra et al., 2021). Bone marrow mesenchymal stem cells treated with SFI retained the function of sinoatrial node in rabbits with sinoatrial node syndrome, improved the expression of HCN4 gene and gap junction proteins (Cx43 and Cx45), and significantly upregulated the expression of cAMP in sinoatrial node (Chen Q. et al., 2022).

In addition, SFI has certain pharmacological effects on nervous system, respiratory system and digestive system. For example, SFI has a protective effect on lipopolysaccharide-induced septic shock in rabbits (Liu X. et al., 2019). It can reduce bile duct injury in rats with acute obstructive cholangitis (Tan et al., 2019) and increase the level of acetylcholine in acute liver injury in septic young rats (Wu et al., 2022). It also has a protective effect on lung and intestinal epithelial injury in mice with acute gastrointestinal injury (Zheng et al., 2022).

5 Clinical trial of SFI in CVDs

There are many clinical trials related to SFI, and nine clinical trials have been conducted to study its role in the treatment of CVDs. These clinical trials have demonstrated that SFI can improve cardiac function and corresponding indicators in patients with CVDs, including heart failure, myocardial infarction, cardiac arrest after resuscitation, coronary syndrome, coronary heart disease and other diseases (Table 1).

5.1 The effect of SFI in patients with acute heart failure

Infusion of SFI in 80 patients with acute heart failure can improve cardiac function, clinical symptoms and quality of life (Wang et al., 2019b). Fifty patients with acute decompensated heart failure were treated with combination therapy. Compared with simple infusion of levosimendan, the improvement of hemodynamic parameters including CI, cardiac output and stroke volume index was more significant, especially in patients with acute decompensated heart failure with hypotension (Li M. et al., 2022).

5.2 The effect of SFI in patients with chronic heart failure

SFI was used to treat 80 patients with acute exacerbation of chronic heart failure, which could improve the symptoms, quality of life, exercise tolerance, improve left ventricular ejective fraction (LVEF), reduce left ventricular end diastolic diameter (LVED), plasma brain natriuretic peptide (BNP) and cytokine Fas, TNF-α, IL-6 levels, reduce mortality and readmission rate (Liu et al., 2015). SFI was administered to 171 patients suffering from chronic heart failure on the basis of Western medicine. Compared with Western medicine alone, it could reduce the all-cause mortality by 30.99%, increase the 6-min walking distance (6MWT) and improve the quality of life (Wang X. et al., 2017). Patients with coronary heart disease complicated with chronic heart failure were treated

with SFI and furosemide injection for 7 days. The effect was better than that of furosemide injection alone in improving cardiac function, LVEF, N-terminal B-type natriuretic peptide (NT-proBNP), TNF-α, IL-6 (Gao et al., 2021). For 7 days, SFI and sodium nitroprusside were administered intravenously to 91 patients who had coronary heart disease and chronic heart failure. The effect was better than that of sodium nitroprusside injection alone in improving cardiac function, LVEF, NT-proBNP and BNP (Guo et al., 2021).

5.3 The effect of SFI in patients with other CVDs

Infusion of SFI 1h before coronary angiography in 74 patients with coronary syndrome undergoing percutaneous coronary intervention (PCI) significantly reduced the level of neutrophil gelatinase-associated lipocalin (NGAL) in urine and effectively prevent contrast-induced acute kidney injury (Guo et al., 2017). SFI was used to treat patients with ST-segment elevation myocardial infarction before PCI and maintained for 5 days after PCI. Compared with patients treated with placebo, SFI reduced the area of myocardial infarction (Wang X. et al., 2021). A total of 492 cardiac arrest patients received bi-daily intravenous SFI infusions over a span of 28 days. The 28-day and 90-day survival rates were improved, the mechanical ventilation time and hospitalization time were shortened, and the recovery of spontaneous circulation after cardiac arrest was effectively improved (Zhang et al., 2017).

6 Concluding remarks and future perspectives

The results of numerous research studies in the past have demonstrated that SFI exerts varying degrees of therapeutic effects on various types of CVDs, such as heart failure, myocardial hypertrophy, myocardial ischemia, cardiac arrest, arrhythmia, and so forth. SFI plays a therapeutic role through multiple different targets, such as TGF-Smads, PI3K-Akt, eNOS-Akt pathway, and so on. SFI has been used in China for more than 30 years. It is a commonly used drug for clinical treatment of CVDs. No serious adverse reactions have been found so far.

In general, SFI is a promising drug for the treatment of CVDs. However, SFI has the characteristics of multi-component, multi-target and multi-pathway, which increases the difficulty of research. There is still a lack of in-depth study on the mechanism of SFI. In addition, large-scale, high-quality, multi-center clinical trials are needed to determine the comparison of SFI with traditional CVDs treatment regimens.

Author contributions

F-FX: Writing–original draft. X-FX: Writing–original draft. H-YH: Writing–original draft. R-ST: Writing–review and editing. CP: Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Yusof Kamisah,
Faculty of Medicine Universiti Kebangaan Malaysia, Malaysia

REVIEWED BY
Kang Liu,
China Pharmaceutical University, China
Benneth Ben-Azu,
Delta State University, Nigeria

*CORRESPONDENCE
Ling Tu,
2836712461@qq.com

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The protective role of ginsenoside Rg3 in heart diseases and mental disorders

Lili Shi[1,2], Jinlan Luo[1,2], Xiupan Wei[3], Xizhen Xu[2,4] and Ling Tu[1,2]*

[1]Department of Geriatric Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [2]Hubei Key Laboratory of Genetics and Molecular Mechanisms of Cardiological Disorders, Wuhan, China, [3]Department of Rehabilitation Medicine, Zhongda Hospital, Southeast University, Nanjing, China, [4]Division of Cardiology and Department of Internal Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Ginsenoside Rg3, a compound derived from *Panax ginseng* C. A. Mey., is increasingly recognized for its wide range of pharmacological effects. Under the worldwide healthcare challenges posed by heart diseases, Rg3 stands out as a key subject in modern research on Chinese herbal medicine, offering a novel approach to therapy. Mental illnesses are significant contributors to global disease mortality, and there is a well-established correlation between cardiac and psychiatric conditions. This connection is primarily due to dysfunctions in the sympathetic-adrenomedullary system (SAM), the hypothalamic-pituitary-adrenal axis, inflammation, oxidative stress, and brain-derived neurotrophic factor impairment. This review provides an in-depth analysis of Rg3's therapeutic benefits and its pharmacological actions in treating cardiac and mental health disorders respectively. Highlighting its potential for the management of these conditions, Rg3 emerges as a promising, multifunctional therapeutic agent.

KEYWORDS

ginsenoside Rg3, heart diseases, mental disorders, heart failure, depression

1 Introduction

Despite extensive research over the years, both heart diseases and mental disorders continue to exert significant pressure on global healthcare systems. Heart diseases maintain their status as the leading cause of death globally (Liu and Miao, 2022; Lupisella et al., 2022). The pharmacological landscape for heart diseases is diverse, encompassing a range of drugs. However, these treatments largely fail to restore cardiac function fundamentally and are frequently associated with side effects.

At the same time, mental illness significantly contributes to the global disease burden. A recent meta-analysis reveals that 14.3% of global deaths annually, equivalent to roughly eight million fatalities, are linked to mental disorders (Walker et al., 2015). Individuals with severe mental illnesses, such as schizophrenia, bipolar disorder, and major depressive disorder (MDD), face a mortality rate of two to three times higher than the average population. The elevated rate corresponds to a reduced life expectancy of 10–25 years (Correll et al., 2015). However, issues associated with treatment discontinuation and ineffectiveness are prevalent.

Up to now, numerous studies have established a strong link between cardiac diseases and psychiatric conditions. It is frequently observed that individuals with cardiac ailments often experience psychiatric disturbances (Piepenburg et al., 2019). Inversely, those with mental disorders appear to have a higher risk of developing heart diseases (Hagi et al., 2021).

Several biological mechanisms are suggested to clarify the association between mental disorders and cardiac events. Mental disorders are linked to dysfunctions in the sympathetic-adrenomedullary system (SAM), and the hypothalamic-pituitary-adrenal (HPA) axis, as well as to inflammation, oxidative stress, and impairments in the brain-derived neurotrophic factor (BDNF) system. All these physiological processes play significant roles in the onset and progression of cardiac diseases.

Recently, the field of Chinese herbal medicine has captured the interest of the scientific community, owing to its extensive range of pharmacological properties and a lower incidence of adverse side effects. Particularly, the effects of ginsenoside Rg3 (Rg3) in cardiac and mental diseases have gained more and more attention.

Consequently, in this review, we provide a detailed investigation of the therapeutic effects and pharmacological action of Rg3 in addressing heart and mental disorders (Table 1). Based on the association between heart and mental disorders, we aim to provide prospects on the potential effects and mechanisms of Rg3 in the comorbid conditions.

2 Origin and structure of Rg3

Rg3 is a constituent of ginsenosides extracted from *Panax ginseng* C. A. Mey. (Nakhjavani et al., 2020). Key ginsenoside constituents, specifically Rb1, Rb2, and Rd, possess the capacity for enzymatic transformation into Rg3 (Lee H. et al., 2020). Rg3 is categorized into two distinct stereoisomers based on its unique spatial configurations at the C20 position: 20(R)-Rg3, as illustrated in Figure 1, and 20(S)-Rg3, depicted in Figure 2. Research findings have underscored the pivotal role of Rg3 across diverse domains, encompassing its involvement in anti-aging mechanisms, anticancer properties, bone development, cellular differentiation, neuroprotection, and cardiac function (Lee et al., 2019; Zarneshan et al., 2022).

3 The relationships between heart diseases and mental disorders

3.1 Mental disorders induce the occurrence of heart diseases

Recent meta-analytic studies have shown that mental illness is a high risk for cardiac events (Zarneshan et al., 2022). In addition, a Mendelian randomization analysis revealed a significant genetic association between MDD and coronary artery disease (CAD). Specifically, the analysis indicated that for each one-unit increase in the natural logarithm of odds of MDD, the odds ratio for CAD was 1.16 (95% confidence interval: 1.05 to 1.29; $p = 0.0047$) (Tang et al., 2020). Recent observational research has indicated a correlation between genetic predispositions for schizophrenia and notable changes in cardiac structure. These alterations have been found to potentially aggravate cardiac health outcomes (Pillinger et al., 2023). Mental health disorders, such as MDD, anxiety, and stress-related conditions, may contribute to behaviors including smoking, inactivity, and drinking adversely affecting cardiac function.

3.2 Heart diseases promote the development of mental disorders

Clinically, a subset of patients with heart diseases commonly experience co-occurring mental disorders, specifically anxiety and depression. Multiple meta-analyses have demonstrated that individuals diagnosed with heart failure (HF) are at an elevated risk of experiencing depression (Hagi et al., 2021). Certain drugs used in managing heart diseases, including beta-blockers, have the potential to initiate or aggravate symptoms associated with anxiety and depression (Bornand et al., 2022; Carnovale et al., 2023). Additionally, managing heart conditions, including HF, entails a prolonged treatment course that can result in significant financial strain for patients and create a disparity between hospitalization needs and employment stability. This significantly heightens the risk of developing mental disorders in individuals with heart diseases.

3.3 The major mechanisms linking heart diseases and mental disorders

3.3.1 Inflammation

Inflammatory processes are associated with the initiation and progression of mental disorders (Ben-Azu et al., 2022a; Ben-Azu et al., 2023). Mental disorders may enhance the expression of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which are associated with endothelial dysfunction and metabolic alterations (Vaccarino et al., 2018; Misiak et al., 2022; Tsioufis et al., 2022; Chen et al., 2023). Inflammation, vascular dysfunction, and metabolic abnormalities frequently contribute to the pathogenesis of heart diseases. Furthermore, inflammatory processes within heart tissue can result in elevated blood levels of pro-inflammatory cytokines and other acute-phase reactants (Hartupee and Mann, 2017). Inflammatory factors may lead to the upregulation of indoleamine 2,3-dioxygenase (IDO), diverting tryptophan (TRP) into the kynurenine (KYN) pathway, potentially reducing serotonin (5-HT) synthesis and contributing to the onset of mental disorders, including depression. 5-HT functions as a neurotransmitter in the central nervous system, a blood factor, and a neurohormone that regulates the function of various peripheral organs (De Deurwaerdere and Di Giovanni, 2020). A deficiency in 5-HT increases susceptibility to social defeat stress and impairs responses to antidepressants (Sachs et al., 2015). Additionally, pro-inflammatory cytokines are associated with a marked decrease in both BDNF gene and protein expression (Zhang et al., 2014). BDNF is crucial for the plasticity of glutamatergic and gamma aminobutyric acid (GABA)ergic synapses and is intimately linked to severe mental illnesses (Colucci-D'Amato et al., 2020). Interestingly, animal studies have demonstrated that in post-myocardial infarction, BDNF expression is upregulated through neuronal signaling originating from the heart. This upregulation serves to shield the myocardium from ischemic damage, thereby exerting a protective effect against cardiac remodeling (Cannavo et al., 2023). Furthermore, studies have noted lower BDNF levels in patients with HF when compared to healthy controls (Xie et al., 2023; Wang et al., 2024). BDNF plays a crucial role in supporting the survival of endothelial cells during the development of the cardiovascular system (Kermani and Hempstead, 2019).

TABLE 1 Summary of effects of Rg3 on heart and mental conditions.

Disease	Model	Type	Treatment (dose, duration)		Described effects and mechanisms	References (PMID)
			Animal	Cell		
HF	TAC-mice	20(R)-Rg3	20 mg/kg	10 μM	Improving cardiac function, inhibiting cardiomyocyte hypertrophy and ER stress via enhancing SUMOylation of SERCA2a	34,428,586
	ISO-HL-1 cell		4 weeks	24 h		
	TAC-mice	—	10 or 20 mg/kg	10 μM	Modulating glucose metabolism and insulin resistance through activation of the AMPK pathway	35,509,823
	Insulin-H9C2		4 weeks	24 h		
	TAC-mice	—	10 or 20 mg/kg	—	Regulating pyruvate metabolism and sustaining glucose homeostasis in cardiac tissue through maintaining the PDHc activity	36,572,672
			4 weeks			
	CAL-mice	20(S)-Rg3	7.5, 15 or 30 mg/kg	1, 5 or 25 μM	Inhibiting myocardial fibrosis via the ACY1/TGFβ1/Smad3 signaling pathway	34916608
	Ang II-CFs		2 weeks	24 h		
	LAD-rat	—	10, 20 or 30 mg/kg	10 μm	Activating mitophagy via the ULK1/FUNDC1 pathway	37,659,296
	H9C2		4 weeks	20 h		
MI	CAL-rat	—	30 mg/kg/day	—	Inhibiting the inflammatory response via the NF-κB pathway	33193843
			1 week			
	ISO-mice	—	5 mg/kg	—	Upregulating autophagy process through the AMPK signaling pathway	32420095
			2 days			
	LAD-mice	20(S)-Rg3	20 or 40 mg/kg	5, 10 or 20 μM	Alleviating myocardial fibrosis through the TGFBR1 signaling pathway	38107395
	TGFβ1-CFs		4 weeks			
MIRI	Rat	—	5 or 20 mg/kg	—	Antiapoptosis and anti-inflammation	28105061
			7 days			
	Rat	—	0.5 mg	10 nM	Antifibrosis, inhibiting oxidative stress and inflammation via SIRT1/PGC1-α/Nrf and IκBα/NF-κB signal pathway	31,783,047
	H9C2			24 h		
	Rat	—	60 mg/kg	10 mM	Inhibiting apoptosis and oxidative stress via Akt/eNOS signaling and the Bcl2/Bax pathway	25,672,441
	NRCs			24 h		
Cardiotoxicity	ADM-rats	—	10, 20 or 40 mg/kg	1, 10 or 100 μM	Inhibiting oxidative stress via activation of the Nrf2-ARE pathway through the activation of Akt	26,321,736
	CMEC		2 weeks	24 h		
	Mice	—	10, 40 or 80 mg/kg	60 μM	Inhabiting apoptosis and oxidative stress through the miR-128–3p/MDM4 axis	37,990,515
	H9C2		4 weeks	25 h		
DCM	Mice	—	25, 50 or 100 mg/kg	5, 10 or 20 μM	Modulating glucose and lipid metabolism by directly binding to PPAR-γ and activation of the adiponectin pathway	38,069,059
	H9C2		12 weeks	48 h		
	3T3-L1					

(Continued on following page)

TABLE 1 (*Continued*) Summary of effects of Rg3 on heart and mental conditions.

Disease	Model	Type	Treatment (dose, duration)		Described effects and mechanisms	References (PMID)
			Animal	Cell		
MDD	LPS-mice	—	20 or 40 mg/kg 3 days	—	Inhibition of neuroinflammatory disturbance and the regulation of TRP-KYN metabolism	28,762,741
	Mice	—	10 or 20 mg/kg 2 weeks	—	Promotion of the BDNF signaling pathway	28,013,484
	Mice NMDA-HT22	—	50, 100 or 150 mg/kg 4 weeks	1, 5 or 10 μM	Recovering proliferation and inhibiting apoptosis via CREB and BDNF signaling pathway	28,461,003
Anxiety	*Xenopus* oocytes	20(S)-Rg3	—	100 μM	Regulating $GABA_A$ receptor channel activity	23,499,684
PTSD	Rat	—	25 or 50 mg/kg 2 weeks	—	Regulating the HPA axis and activating the BDNF-TrkB pathway	35,982,366
ADHD	Mice SH-SY5Y	YY162(including 20(R)-Rg3 and 20(S)-Rg3)	200 mg/kg 2 weeks	100 μg/mL 50 h	Inhibiting oxidative stress	24,394,491

TAC, transverse aortic constriction; ISO, isoproterenol; ER, endoplasmic reticulum; SERCA2a, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; AMPK, AMP-activated protein kinase; PDHc, pyruvate dehydrogenase complex; CAL, coronary artery ligation; AngII, angiotensin II; ACY1, aminoacylase-1; TGFβ1, transforming growth factor-β 1; LAD, left anterior descending coronary artery ligation; ULK1, Unc51-like-kinase 1; FUNDC1, FUN14 domain-containing protein 1; NFκB, nuclear factor κB; TGFBR1, transforming growth factor beta receptor 1; PGC1-α, peroxisome proliferators-activated receptor γ coactivator l alpha; IκBα, inhibitor of kappa B alpha; Nrf2, nuclear factor erythroid 2-related Factor 2; Akt, protein kinase B; eNOS, endothelial nitric oxide synthase; Bcl-2, B cell lymphoma-2; Bax, Bcl2-associated X protein; ARE, antioxidant response element; MDM4, double minute 4 protein; PPAR-γ, peroxisome proliferator-activated receptor γ; TRP, tryptophan; KYN, kynurenine; BDNF, brain-derived neurotrophic factor; CREB, cyclic adenosine monophosphate response element binding protein; HPA, hypothalamic-pituitary-adrenal; TrkB, tropomyosin-related kinase B; LPS, lipopolysaccharide; HT22, murine hippocampal neuronal; NMDA, n-methyl-d-aspartate; HF, heart failure; MI, myocardial infarction; MIRI, myocardial ischemia-reperfusion injury; MDD, major depressive disorder; DCM, diabetic cardiomyopathy; PTSD, post-traumatic stress disorder; ADHD, attention deficit hyperactivity disorder.

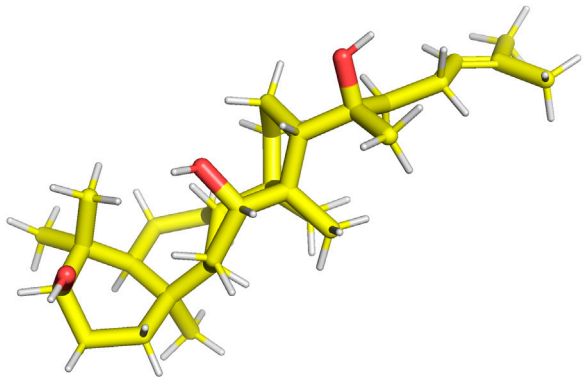

FIGURE 1
Structure of 20(R)-Rg3.

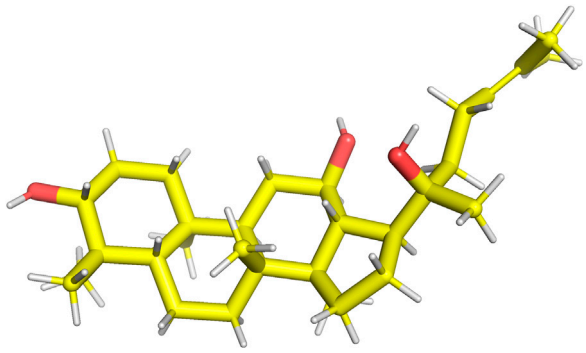

FIGURE 2
Structure of 20(S)-Rg3.

3.3.2 HPA axis

The HPA axis serves as a critical component of the neuroendocrine system, orchestrating responses to both internal and external stressors. Various mental disorders have been shown to trigger the activation of the HPA axis (Sur and Lee, 2022b; Emudainohwo et al., 2023; Menke, 2024). This activation leads to an upsurge in cortisol synthesis within the adrenal cortex, along with enhanced production of adrenaline and noradrenaline in the adrenal medulla (Ugwu et al., 2022). Hypercortisolemia, commonly observed in mental disorders, can lead to escalated steroid production, elevated blood pressure, and an increase in visceral fat (García-Eguren et al., 2019; Favero et al., 2021; Teng et al., 2021). These changes significantly heighten the risk of heart diseases. Elevated cortisol levels following stress are directly linked to the hypertrophy of cardiomyocytes and cardiac remodeling (Opinion et al., 2023). Additionally, plasma cortisol concentrations have been identified as an independent risk factor for cardiac events and mortality (Crawford et al., 2019; Kim et al., 2022). Furthermore, the stimulation of the HPA axis results in increased aldosterone levels. Studies have shown that stress-related aldosterone activity has been linked to

hypertension, myocardial necrosis, and fibrosis. Additionally, a rise in aldosterone has been associated with increased insulin resistance, oxidative stress, and pro-inflammatory responses (Bothou et al., 2020; Tsai et al., 2021; Campana et al., 2022). In chronic HF, elevated serum cortisol levels have been identified as an independent predictor of increased mortality risk (Güder et al., 2007). Moreover, increased cortisol levels may exacerbate the brain's vulnerability to oxidative stress, potentially leading to detrimental effects on neurobehavioral health. Stress-induced cortisol secretion may lower brain 5-HT and BDNF function, potentially leading to the onset of depressive symptoms and anxiety (Bhagwagar et al., 2002; Motta et al., 2021).

3.3.3 SAM

Negative psychological states can activate the SAM system, resulting in elevated catecholamine levels. Catecholamines, including epinephrine, norepinephrine, and dopamine, are tyrosine-derived hormones and neurotransmitters primarily synthesized in the adrenal medulla, sympathetic nerves, and brain. Elevated catecholamine levels can induce vasoconstriction, leading to cardiac injury, HF, myocardial ischemia, and necrosis (Szatko et al., 2023). Persistent catecholamine elevation may cause myocardial calcium overload in cytosolic and mitochondrial compartments, trigger oxidative stress, increase mitochondrial permeability, and cell death (Szatko et al., 2023). In patients with HF, there is an activation of the neuroendocrine systems, particularly the sympathetic nervous system and the renin-angiotensin-aldosterone system, leading to elevated levels of neurohormones such as catecholamines (Manolis et al., 2023). Dopamine, an important catecholamine, plays a crucial role in regulating various mental and physical functions, including anxiety, fear, attention deficit hyperactivity disorder (ADHD), and schizophrenia (Jayanti et al., 2023). Additionally, norepinephrine, another key catecholamine, acts as a neurotransmitter and is essential in mediating physiological and behavioral responses to stress (Schmidt et al., 2019).

4 Pharmacological action of Rg3 in heart diseases

4.1 HF

HF is a complicated condition that leads to aggressive hazards to human health. The common therapeutic regimens for HF patients predominantly involve diuretics, vasoactive drugs, and other pharmaceutical approaches (Truby and Rogers, 2020). Medications such as angiotensin-converting enzyme inhibitors (ACEIs), beta-blockers, mineralocorticoid receptor antagonists, and angiotensin receptor-neprilysin inhibitors can partially alleviate HF symptoms. However, these treatments are often insufficient in significantly reducing rehospitalization rates and mortality, even when patients adhere to established guidelines (Lai et al., 2022). Moreover, traditional medication can lead to adverse effects, including hypotension, hypokalemia, and renal function impairment (Hubers and Brown, 2016; Marciniak and Serebruany, 2019; Rossello et al., 2022). Consequently, there is a pressing need to explore new therapeutic agents to enhance survival rates and improve the quality of life for HF patients. Rg3 has emerged as a promising treatment option in HF, offering protective benefits such as promoting cardiomyocyte relaxation, enhancing mitochondrial structure and function, regulating metabolism, reducing cardiac fibrosis, and preventing cell apoptosis.

Cardiac sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), a crucial protein in the Ca^{2+} cycle of cardiomyocytes, is involved in Ca^{2+} reuptake into the cytoplasm and subsequent transport to the endoplasmic reticulum (ER), ultimately promoting cardiomyocytes relaxation (Liu et al., 2021; Chen et al., 2022). SUMO binds to certain lysine sites on SERCA2a, forming the SERCA2a/SUMO complex. Notably, HF patients and mice exhibited significantly reduced levels of SERCA2a SUMOylation (Mendler et al., 2016). However, Rg3 treatment could increase the SUMOylation of SERCA2a, further increase intracellular Ca^{2+} cycle protein levels, suppress ER stress and prevent reaction oxygen species (ROS) generation, thus improving cardiac function and inhibiting cardiomyocyte hypertrophy in transverse aortic constriction (TAC) induced HF mice (Liu et al., 2021).

Moreover, Rg3 could improve disordered mitochondrial ultrastructure, functions such as ATP production and spare respiratory capacity, and regulate glucose uptake, and myocardial insulin resistance (Ni et al., 2022a). Rg3 regulated glucose uptake and myocardial insulin resistance through the activation of insulin receptor substrate (IRS)-phosphoinositide 3 kinase (PI3K)-protein kinase B (Akt) signaling pathway (Ni et al., 2022a). Several pieces of experimental evidence suggested that the decoupling of glucose oxidation to glycolysis may be the cause of unaltered or reduced pyruvate oxidation in mitochondria in HF (Pound et al., 2009; Bertero and Maack, 2018; Fillmore et al., 2018). Pyruvate dehydrogenase complex (PDHc) plays a pivotal role in regulating mitochondrial pyruvate metabolism with dihydrolipoamide dehydrogenase (DLD), serving as a crucial part of PDHc (Staretz-Chacham et al., 2021; Kim et al., 2023). P300 and tat-interacting protein 60 (TIP60) are recognized as 2-hydroxyisobutyryltransferases that could regulate the activity of PDHc (Sabari et al., 2017; Huang et al., 2018). In HF, the 2-hydroxyisobutylation of DLD was significantly upregulated, resulting from the downregulation of PDHc activity. However, Rg3 can lower the 2-hydroxyisobutylation levels of DLD and maintain the PDHc activity by suppressing the acyltransferase activity of P300, further regulating pyruvate metabolism and sustaining glucose homeostasis in cardiac tissue, consequently, improving cardiac function (Ni et al., 2022b).

Another study demonstrated that in HF mice, Rg3 administration increased the expression of aminoacylase-1 (ACY1) and inhibited cardiac fibrosis, thereby, ameliorating heart function through the ACY1-mediated transforming growth factor-β1 (TGF-β1)/Smad3 pathway. In this study, metoprolol served as the positive control. Both Rg3 and metoprolol significantly enhanced cardiac function. Notably, the impact of Rg3 at high dosage on HF was found to be comparable to that of metoprolol. Furthermore, in murine cardiac fibroblasts, the intervention of angiotensin II (AngII) resulted in an upregulation of collagen 1, collagen 3, α-smooth muscle actin, tissue inhibitor of metalloproteinases 1, and the TGF-β1/Smad3 signaling pathway, which could be reversed in case of overexpressing ACY1 and Rg3 administration (Lai et al., 2022). Additionally, Baoyuan decoction, a mixture of several Chinese herbs, consists of *Astragalus membranaceus* (Fisch.) Bunge,

Glycyrrhiza uralensis Fisch., *Cinnamomum cassia* Presl and *P. ginseng* C. A. Mey. (Wang et al., 2020). A study demonstrated that its active component Rg3 effectively suppressed cardiomyocyte apoptosis via angiotensin type 1 receptor (AT1)-cardiac ankyrin repeat protein (CARP) signaling pathway (Wang et al., 2020). CARP, a downstream protein of AT1 can accelerate apoptosis by activating the P53-mitochondrial apoptotic pathway (Shen et al., 2015). In another study, Rg3 could be an Unc51-like-kinase 1 (ULK1) regulator to facilitate FUN14 domain-containing protein 1 (FUNDC1)-mediated mitophagy, thus restoring mitochondria homeostasis and energy metabolism in HF (Wang et al., 2023). Additionally, trimetazidine was selected as the positive control. The findings indicated that the efficacy of trimetazidine was equivalent to that of a medium dose of Rg3 in rats with HF. Furthermore, a high dose of Rg3 exhibited the most pronounced therapeutic effectiveness (Wang et al., 2023).

4.2 Myocardial infarction (MI) and myocardial ischemia-reperfusion injury (MIRI)

MI and MIRI are global health issues characterized by high incidence and mortality rates across different countries. The pathophysiological basis of MI is closely related to mitochondria dysfunction, the depletion of endogenous antioxidants, and lipid peroxidation (Wang and Kang, 2021; Li D. et al., 2023; Cai et al., 2023). Following an acute MI event, clinical intervention typically involves thrombolysis, percutaneous coronary intervention (PCI), and coronary artery bypass grafting (Doenst et al., 2019; Mackman et al., 2020; Sabatine and Braunwald, 2021). These therapeutic strategies are effective in restoring blood flow, alleviating pain, and minimizing myocardial damage. Although these treatments have contributed to a substantial decrease in mortality rates, a subset of patients may experience complications, including hemorrhage and MIRI (Reed et al., 2017; McCarthy et al., 2018; Doenst et al., 2019). It has been observed that an incidence of 10%–25% of recurrent acute MI and a hospital death rate of 6%–14% among MIRI patients following PCI therapy (Wu et al., 2019). In MIRI, several medications, including antiplatelet drugs may extend survival times. However, a significant proportion of these treatments are associated with adverse effects such as bleeding and suboptimal targeting efficiency (Zhang et al., 2022). Additionally, some studies suggest that conventional antiplatelet medications do not markedly improve clinical symptoms (Li et al., 2021). Thus, combining with traditional Chinese medicine may be a promising therapeutic method under virtue of fewer adverse reactions (Tu et al., 2020).

A few studies in animals have proved that Rg3 exerted protective action on MI by promoting mitophagy, and inhibiting apoptosis, myocardial fibrosis as well as inflammation. A study showed that in isoproterenol (ISO)-induced MI mice, Rg3 pretreatment could decrease ROS content in the myocardium, promote autophagy via the AMP-activated protein kinase (AMPK)/acetyl CoA carboxylase (ACC) signal pathway, and inhibit apoptosis (Sun et al., 2020). Another study displayed that in MI rats, Rg3 downregulated the levels of pro-inflammatory cytokines in serum and cardiac tissue such as TNF-α, interleukin-1β (IL-1β), and IL-6 and increased the levels of anti-inflammatory cytokine interleukin-10 (IL-10). The anti-inflammation mechanism of Rg3 is related to the increased expression levels of sirtuin 1 (SIRT1) and decreased expression of p-P65 (Tu et al., 2020). P65 is a crucial part of the nuclear factor κB (NF-κB). SIRT1-deacetylated P65 inhibits NF-κB activation by impeding its nuclear translocation and further restrains the transcription of TNF-α, IL-6, and other inflammatory genes downstream of NF-κB, thereby mitigating the inflammatory response (Chen et al., 2016; Tu et al., 2020). A study showed in MI mice and TGFβ1-stimulated primary cardiac fibroblasts (CFs), Rg3 suppressed CF proliferation along with collagen deposition by inactivation of transforming growth factor beta receptor 1 (TGFBR1)/Smads signaling dose-dependently (Xu et al., 2023). TGFBR1 overexpression partially abolished Rg3's inhibition on Smad2/Smad3 activation, CFs growth, together with collagen production. In this study, captopril was employed as the positive control. The results indicated that Rg3 improved cardiac function in a dose-dependent manner, with the high dose of Rg3 demonstrating effects comparable to those of captopril (Xu et al., 2023).

In addition, Rg3 exerted a beneficial role in MIRI via inhibiting inflammation, apoptosis, oxidative stress, and attenuating cardiac fibrosis. In MIRI rat models, the use of Rg3 downregulated significantly the levels of inflammatory cytokines in plasma by inhibiting inhibitor of kappa B alpha (IκBα)/NF-κB signal pathway and further improved cardiac function (Zhang et al., 2016; Li et al., 2020). Moreover, Rg3 ameliorated myocardial collagen deposition via the blockage of the TGF-β/Smad signaling pathway and exerted an anti-apoptotic effect via the Akt/endothelial nitric oxide synthase (eNOS) signaling pathway and the B cell lymphoma-2 (Bcl-2)/Bcl2-associated X protein (Bax) pathway (Wang Y. et al., 2015; Li et al., 2020). Hypoxia/reoxygenation (H/R) is a typical approach to generate MIRI injury in cardiocytes (Du et al., 2023). Rg3 administration strongly targeted FoxO3a to decrease the ROS content via the SIRT1/peroxisome proliferators-activated receptor γ coactivator-1α (PGC1-α)/nuclear factor erythroid 2-related factor (Nrf) pathway, and inhibit inflammation through IκBα/NF-κB signal pathway in H9C2 cells induced by H/R (Li et al., 2020).

4.3 Cardiotoxicity

Cardiotoxicity is an essential consideration in evaluating whether drugs can be marketed during preclinical trials and is a major reason for treatment withdrawal even after approval (Coelho et al., 2017). Even though some drugs have been used, cardiotoxicity confines their use in clinical practice. Nowadays, a good chunk of drugs, mostly anti-cancer medicine, usually lead to cardiotoxicity. Cancer therapy-induced cardiotoxicity significantly impacts patients mortality, long-term prognosis, and overall quality of life. Anthracyclines play an essential role in cancer therapy, including doxorubicin (DOX), epirubicin, daunorubicin, actinomycin, and valrubicin, have been widely applied to treat multiple types of cancer (Sawicki et al., 2021; Qu et al., 2022). However, their clinical application has been constrained by diverse adverse actions, including cardiotoxicity (Qu et al., 2022). DOX-induced cardiotoxicity involves a complex mechanism, including excessive ROS production, alterations in cell membrane integrity, and apoptosis (Upadhyay et al., 2020; Rawat et al., 2021; Tai et al.,

2023). Standard treatments for cardiotoxicity encompass ACEIs, angiotensin receptor blockers (ARBs), beta-blockers, and calcium channel blockers. Research has indicated that ACEI/ARB and beta-blockers may have a positive impact on reducing the long-term health risks associated with anthracycline-induced cardiac dysfunction (Livi et al., 2021). However, previous studies have yielded inconsistent conclusions, possibly related to tumor subtypes and staging. Consequently, there is a need for further investigation into novel medications and complementary therapeutic approaches. A substantial array of natural medicines plays a significant role in mitigating drug-induced cardiotoxicity (Qu et al., 2022).

Traditional Chinese medicine has gained increasing attention for the treatment of various diseases. The protective effects of Rg3 on drug-induced cardiotoxicity have been proved in experiments. Rg3 demonstrated a remarkable ability to inhibit the upregulation of ROS and malondialdehyde, in the meantime, promote superoxide dismutase (SOD), and restore the balance of SOD/glutathione peroxidase in DOX-treated cardiac microvascular endothelial cells and rats through activating the Nrf2/antioxidant response element (ARE) and PI3K/Akt pathway (Wang X. et al., 2015). Furthermore, Rg3 could enhance endothelial function, thus playing a protective impact on cardiac function (Geng et al., 2020).

Another study showed the administration of Rg3 could enhance cardiac function in a dose-dependent manner. Specifically, in cardiotoxicity induced by microcystin, Rg3 acted by downregulating miR-128–3p and upregulating the expression of double minute 4 protein (MDM4), thereby mitigating oxidative stress and reducing cell apoptosis (Zhou and Xia, 2023).

4.4 Diabetic cardiomyopathy (DCM)

DCM is a pathophysiological condition induced by diabetes, potentially leading to HF. The diminished performance of the diabetic heart is attributed to multiple factors, including hyperglycemia, elevated fatty acids, and inflammatory cytokines (Dillmann, 2019). Current therapeutic options for DCM primarily comprise sodium-glucose cotransporter 2 inhibitors, glucagon-like peptide-1 receptor agonists, metformin, thiazolidinediones, and dipeptidyl peptidase 4 inhibitors. While clinical trials have confirmed their effectiveness in ameliorating cardiac dysfunction, their ability to fully cure or substantially improve the prognosis of DCM remains limited. Consequently, there is an urgent need to discover novel treatments specifically targeting DCM. A recent research highlights the potential of Rg3 in DCM management (Zhang et al., 2023). Rg3 appears to protect against DCM by modulating glucose and lipid metabolism, achieved through direct binding to peroxisome proliferator-activated receptor γ (PPAR-γ) and stimulating the adiponectin pathway. Additionally, Rg3 reduces proinflammatory cytokines and mitigates mitochondrial dysfunction. In this study, metformin served as a positive control, and varying doses of Rg3 were evaluated in DCM mice models. The findings indicated that high doses of Rg3, similar to metformin, effectively improved body weight, blood glucose, body fat, and serum lipid levels. However, lower doses of Rg3 did not exhibit these benefits. Moreover, high-dose Rg3 significantly decreased serum creatine kinase (CK), creatine kinase-MB (CK-MB), and lactate dehydrogenase (LDH) levels in diabetic mice, indicative of reduced cardiac dysfunction. In contrast, metformin and low-dose Rg3 only partially improved CK and LDH levels. Therefore, high-dose Rg3 demonstrated more comprehensive systemic effects than metformin.

5 Pharmacological action of Rg3 in mental disorders

5.1 MDD

MDD, commonly referred to as depression, is a long-lasting, recurring, and possibly life-threatening psychiatric illness that impacts up to 20% of the global population (Nabavi et al., 2017). It is usually characterized by diminished self-esteem, cognitive and emotional impairments, reduced energy levels, as well as unexplained pain. Depression has been recognized as one of the primary contributors to the global burden of disease (Akpınar and Karadağ, 2022). The management of depression has become a critical issue for humanity. Primary drugs include serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, serotonin modulators, and atypical antidepressants (Chin et al., 2022). Meta-analyses indicate that the efficacy of existing antidepressants is observed in only about half to one-third of patients with depression (Kennedy et al., 2009; Jensen et al., 2023). Moreover, a significant number of patients experience recurrent episodes of depression. Consequently, there is an urgent need to explore and develop new antidepressant medications. Several investigations have demonstrated that Rg3 exhibits a therapeutic effect on depression.

Rg3 could alleviate depression-like behaviors such as anorexia, anhedonia, and decreased social exploration (Kang et al., 2017; You et al., 2017; Zhang et al., 2017). The exact mechanism refers to inhibiting inflammation cytokines such as IL-1β and IL-6, restoring the balance of TRP-KYN metabolism, enhancing cell proliferation, and suppressing apoptosis. In MDD patients and animal models of depression, the TRP-KYN metabolic pathway is over-activated (Parrott et al., 2016). The metabolic processing of neurotoxic KYN initiates depressive-like behavior after peripheral immune activation. However, Rg3 administration could decrease the level of KYN (Kang et al., 2017). Moreover, one extensively supported hypothesis regarding depression is the neurotrophic hypothesis, positing that the pathogenesis of depression is associated with impaired functioning of the BDNF system within the brain (Colucci-D'Amato et al., 2020). BDNF is crucial for neural signal transduction and the facilitation of neuronal plasticity, achieved through its specific binding and subsequent activation of tropomyosin-related kinase B (TrkB) receptors (Moya-Alvarado et al., 2022; Pahlavani, 2023). In the chronic mild stress mouse model, Rg3 markedly enhanced the expression of BDNF and the phosphorylation of cyclic adenosine monophosphate response element binding protein (CREB), thereby mitigating depressive symptoms (You et al., 2017). Meanwhile, fluoxetine was employed as a positive control, revealing that to achieve a comparable antidepressant effect to that of fluoxetine, a higher dosage of Rg3 is necessary (You et al., 2017). Additionally, it was

observed that chronic stress exposure markedly decreased body weight. While fluoxetine was effective in mitigating this weight loss, Rg3 exhibited no significant impact on body weight.

5.2 Anxiety disorders

Anxiety disorders constitute the most prevalent category of mental illness, typically originating before or during early adulthood. Key characteristics encompass excessive fear and anxiety or avoidance of perceived threats that are persistent and impairing (Penninx et al., 2021). In adults, anxiety prevention has been evaluated in a few trials of selective or indicated prevention (Deady et al., 2017). Effective treatments for anxiety disorders are available, which not only alleviate symptoms of anxiety but also enhance overall quality of life and functioning. Both pharmacotherapy and psychotherapy are regarded as primary approaches to managing anxiety disorders. Studies have demonstrated that routine medications are mildly to moderately effective in treating these disorders, although there is a noted variability in response rates (Penninx et al., 2021). Chinese herbs have shown therapeutic effects on anxiety including Rg3.

Rg3 has shown a beneficial role in $GABA_A$ receptor-related anxiety (Lee et al., 2013). $GABA_A$ receptors consist of three subunits (α1, β1, γ2). The $GABA_A$ receptor is responsible for fast inhibitory synaptic transmission. The principal physiological and pharmacological functions of $GABA_A$ receptors encompass the mitigation of anxiety symptoms in patients. Additionally, the γ2 subunit of the $GABA_A$ receptor is pivotal in the control and management of human epilepsy (Lee et al., 2013). In a study, Rg3 exhibited enhancing effects on the GABA-induced inward current (I_{GABA}) with γ2 subunit in a dependent manner. When the γ2 subunit expression ratio was raised, the degree of the Rg3-induced activation of the $GABA_A$ receptor increased (Lee et al., 2013).

5.3 Post-traumatic stress disorder (PTSD)

PTSD is a severe psychiatric condition linked to substantial distress and impaired functional capacity (Bryant et al., 2023). Psychological therapies are established as the primary treatment modality for PTSD (Bisson and Olff, 2021). Numerous systematic reviews have consistently affirmed the efficacy of these therapies in addressing PTSD symptoms (Bisson et al., 2013). Nevertheless, the effectiveness of psychological interventions may fluctuate based on individual factors, including the intensity of PTSD. Consequently, pharmacotherapy plays a crucial role in the symptom management of PTSD patients. While common pharmacological treatments have shown encouraging outcomes in diminishing the severity of PTSD symptoms, their overall efficacy remains moderate, and they may precipitate adverse reactions. Therefore, there is an imperative need for the exploration and development of innovative therapeutic strategies.

Rg3 has been reported to have a role in improving fear memory and spatial memory (Sur and Lee, 2022a). The HPA axis and monoamine imbalance in the medial prefrontal cortex and hippocampus contribute to the pathogenesis of PTSD. However, Rg3 administration could be involved in regulating the HPA axis and BDNF-TrkB pathway (Sur and Lee, 2022a). In rats administered with Rg3, a significant decrease in serum corticosterone and adrenocorticotropic hormone levels was observed, alongside a marked increase in BDNF, TrkB, catecholamine, and 5-HT concentrations. The study utilized paroxetine hydrochloride as a positive control. Compared to paroxetine hydrochloride, Rg3 demonstrated superior effects in behavioral tests. Notably, a dosage of 50 mg/kg of Rg3 was effective in elevating 5-HT levels, while significant differences were observed in the response to paroxetine hydrochloride treatment.

5.4 ADHD

ADHD is a mental disorder that typically emerges in childhood and is characterized by developmentally inappropriate and impairing inattention, motor hyperactivity, and impulsivity, with difficulties often continuing into adulthood. Medication and behavioral therapies are widely recognized and frequently utilized treatments for ADHD, demonstrating substantial efficacy and notable rates of symptom remission. Furthermore, a recent meta-analysis has revealed a significant preference for herbal medicine as an effective treatment option for ADHD (Dutta et al., 2022). An open-label pilot study demonstrated that the combination of omega-3 and Korean red ginseng may improve ADHD symptoms and cognitive functions including attention, memory, and executive function in children with ADHD (Lee J. et al., 2020). Additionally, YY162, consisting of Rg3 extracted from *P. ginseng* C. A. Mey. and *Ginkgo biloba* L. (Ben-Azu et al., 2022b; Asiwe et al., 2023), exerted protective activity on ADHD through modulating oxidative metabolism and the BDNF/TrkB pathway (Nam et al., 2014). Yet, the mechanism of Rg3 on ADHD requires further research and exploration.

6 Rg3 in the coexistence of cardiac diseases and psychiatric disorders

Current investigations into the therapeutic effects and mechanisms of Rg3, especially concerning the comorbidity of heart and mental disorders, are still in their early stages. Nowadays, there is substantial basic research supporting Rg3's efficacy in treating heart or mental disorders separately, based on their relationship, Rg3 may show potential effects in treating their comorbidity (Figure 3).

Rg3 might play a dual preventive role, potentially arresting the progression or aiding the recovery of mental disorders when used in cardiac disease treatments. Its potential mechanisms might involve modulating inflammation and oxidative stress. In cardiac disease management, Rg3 chiefly functions by reducing inflammation and curtailing oxidative stress. Anti-inflammatory action is linked to the regulation of TRP/KYN metabolism, enhancing 5-HT and BDNF levels, and thereby alleviating mental disorders (Kuo et al., 2021; Fellendorf et al., 2022; Gong et al., 2023). MDD is characterized by reduced antioxidant concentrations in plasma (Bhatt et al., 2020). Oxidative stress, characterized by the overproduction of ROS and

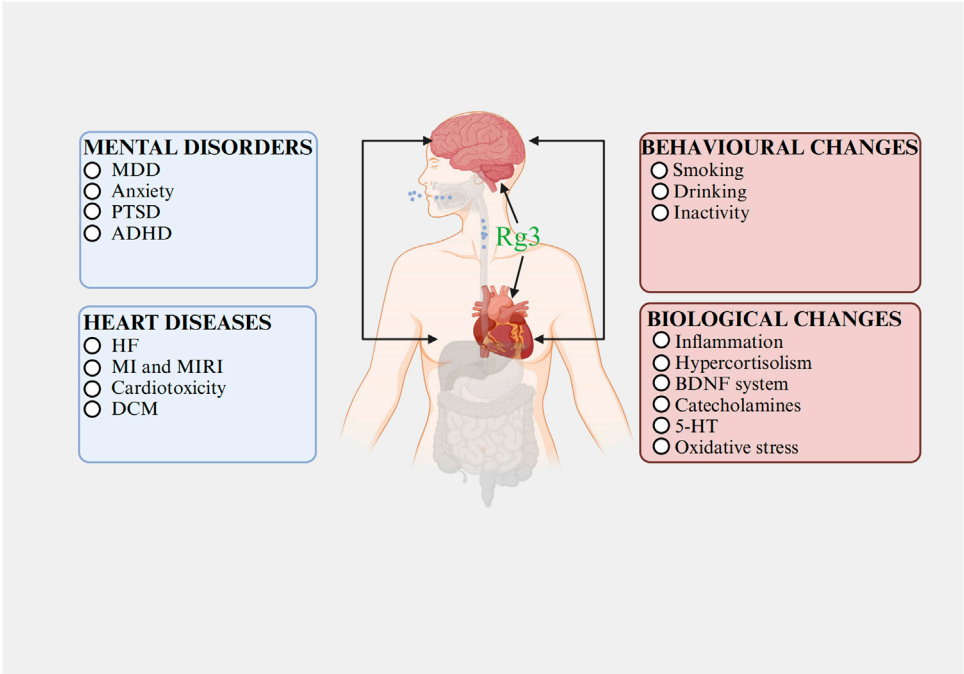

FIGURE 3
The relationships between heart diseases and mental disorders Abbreviations: MDD, major depressive disorder; PTSD, post-traumatic stress disorder; ADHD, attention deficit hyperactivity disorder; HF, heart failure; MI, myocardial infarction; MIRI, myocardial ischemia-reperfusion injury; DCM, diabetic cardiomyopathy; BDNF, brain-derived neurotrophic factor; 5-HT, serotonin. Image created with BioRender.com, with permission.

the depletion of antioxidative defenses, leads to pro-inflammatory signaling and induces cellular apoptosis (Bhatt et al., 2020). Counteracting oxidative stress can therefore diminish inflammation, reduce cellular death, and improve mental health conditions.

Furthermore, in the treatment of mental disorders, Rg3 may also enhance cardiac function. For mental conditions, Rg3 works by suppressing inflammation and HPA axis activity, reducing oxidative stress, boosting BDNF levels, and lowering catecholamine levels. Inflammation and oxidative stress are known to worsen myocardial fibrosis and impair cardiac function. Elevated cortisol levels can directly lead to hypertrophy of cardiomyocytes and cardiac remodeling (Braukyliene et al., 2022). A normal BDNF system is essential for cardiovascular development, while high catecholamine levels can cause vasoconstriction, contributing to cardiac injury, pathological remodeling, heart failure, myocardial ischemia, and necrosis (Du et al., 2021; Agorrody et al., 2022; Tsai et al., 2022). Thus, the mechanism through which Rg3 enhances cardiac function while ameliorating mental disorders may involve modulating inflammation, catecholamine, the BDNF system, the HPA axis, and oxidative stress.

7 Adverse reactions of Rg3

Few studies have shown the side effects of Rg3. Existing clinical investigations of Rg3 at various dosages have not revealed any harmful reaction. The sole adverse observation demonstrated increased but reversible kidney weight in dogs that received 60 mg/kg 20(S)-Rg3 (Gao et al., 2020; Nakhjavani et al., 2020). However, given that Rg3 is extracted from ginseng, high doses of ginseng (15 g/d) will lead to ginseng abuse syndrome. Most symptoms encompass headache, dizziness, breast pain, nausea, asthma, and so on (Siegel, 1979; Deng et al., 2023). Although Rg3 offers a broad spectrum of clinical applications in cancer patients, it should not be abused. Taking the recommended dose of Rg3 will not cause serious adverse reactions. Due to the multiple ingredients of ginseng, the adverse effects of Rg3 are not completely equal to ginseng and need to be explored.

8 Conclusion and perspectives

Recent studies have provided increasing evidence of the broad pharmacological effects of Rg3 in treating heart diseases and mental disorders through a variety of signaling pathways and influencing changes at the transcriptional level (Figure 4; Figure 5).

However, the majority of studies primarily concentrate on *in vitro* cell or animal models, with less emphasis on clinical research. The shenyi capsule, comprising Rg3 monomer, is currently employed in the clinical treatment of cancer patients. Clinical studies have demonstrated that shenyi capsules mitigate the toxicity of platinum-based chemotherapy in non-small-cell lung cancer (NSCLC), including a reduction in platelet toxicity (Xu et al., 2020). Additionally, in a double-blind, randomized, crossover study involving 23 participants, oral administration of Rg3-enriched Korean red ginseng at a dose of 400 mg/day for 7 days

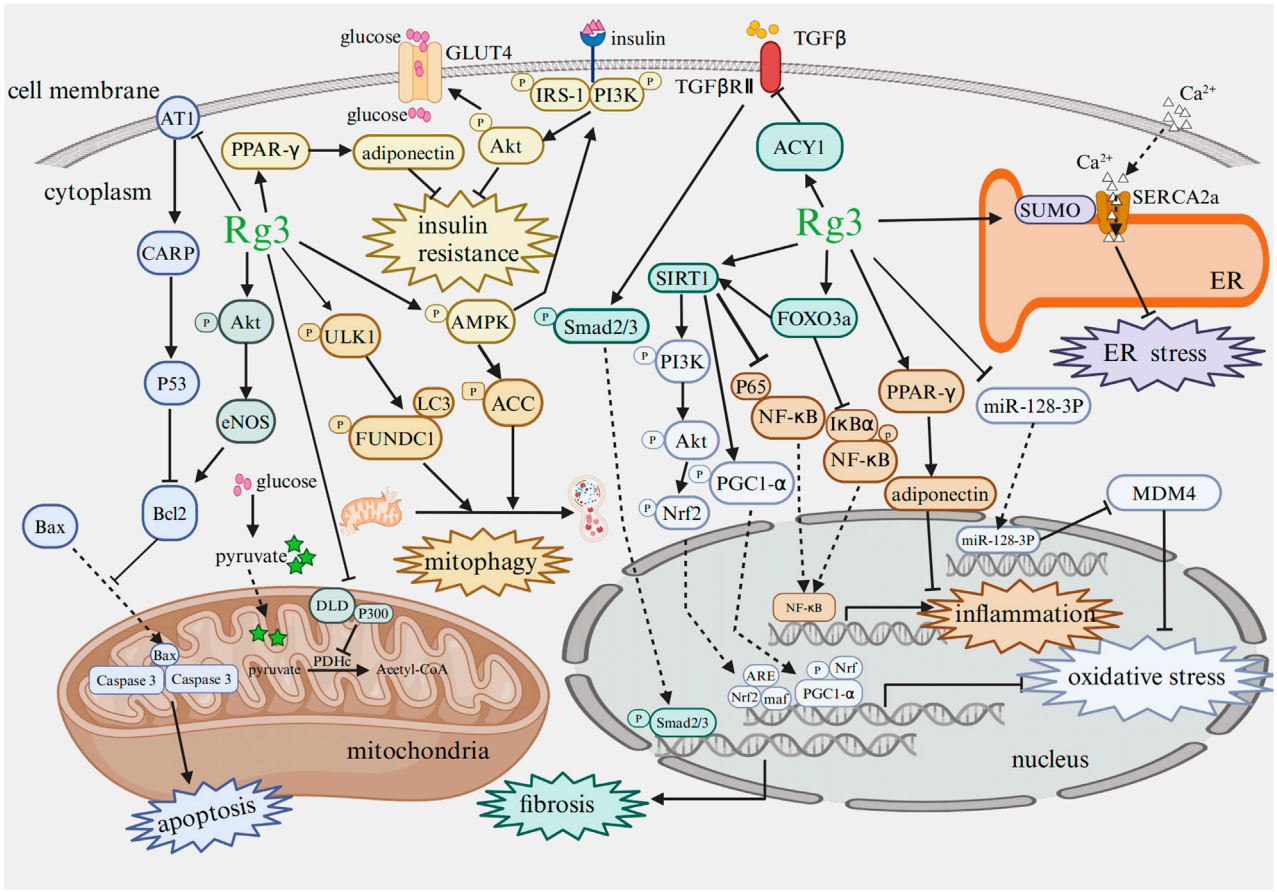

FIGURE 4
Mechanism of Rg3 on cardiac protection Abbreviations: AT1, angiotensin type 1 receptor; CARP, cardiac ankyrin repeat protein; Bcl2, B cell lymphoma-2; Bax, Bcl2-associated X protein; Akt, protein kinase B; eNOS, endothelial nitric oxide synthase; DLD, dihydrolipoamide dehydrogenase; GLUT4, glucose transporter 4; PDHc, pyruvate dehydrogenase complex; ULK1, Unc51-like-kinase 1; FUNDC1, FUN14 domain-containing protein 1; LC3, microtubule-associated protein 1 light chain 3; PI3K, phosphoinositide 3 kinase; IRS, insulin receptor substrate; AMPK, AMP-activated protein kinase; ACC, acetyl CoA carboxylase; Nrf, nuclear factor erythroid 2-related factor; PGC1-α, peroxisome proliferators-activated receptor γ coactivator-1α; PPAR-γ, peroxisome proliferator-activated receptor γ; NF-κB, nuclear factor κB; IκBα, inhibitor of kappa B alpha; ARE, antioxidant response element; maf, musculoaponeurotic fibrosarcoma oncogene homolog; SIRT1, sirtuin 1; FOXO3a, forkhead box O3a; ER, endoplasmic reticulum; SERCA2a, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; MDM4, double minute 4 protein. Image created with BioRender.com, with permission.

demonstrated a decrease in aortic stiffness and central blood pressure (Jovanovski et al., 2014). These findings suggest that Rg3 supplementation may help reduce risk factors for heart diseases such as platelet abnormality, vascular stiffness, and hypertension. However, a clinical study was conducted to recruit NSCLC patients with normal cardiac function. The study showed the treatment group with exclusive administration of Rg3 and the control group with placebo treatment exhibited comparable cardiac functions before and after the therapy (Li D.-R. et al., 2023). Furthermore, an open-label pilot study indicated that a combination of omega-3 and Korean red ginseng, which is rich in Rg3, could potentially alleviate symptoms of ADHD (Lee J. et al., 2020).

However, to date, there is no research exploring the exclusive or combined clinical use of Rg3 for treating heart diseases or mental disorders or the comorbidity of both in humans. The limited clinical research on Rg3 may be attributed to several factors. First, existing studies indicate that a higher concentration of Rg3 is necessary to attain similar therapeutic outcomes as routine drugs (You et al., 2017). Then, the utilization of Rg3 in the clinic is restrictive due to low bioavailability and poor intestinal absorption capability with a percentage of approximately 10% (Xiong et al., 2023). Moreover, common methods for preparing Rg3, such as heat treatment, acid-base treatment, and biological transformation (Fu, 2019; Siddiqi et al., 2020), are hindered by the low natural concentration of Rg3 in ginseng plants (Yan et al., 2019). This results in the inability of most methods to achieve large-scale production of Rg3. Finally, there is a necessity to increase awareness and understanding of Rg3's therapeutic potential in mental disorders and its possible synergistic use in cases of mental disorders co-occurring with heart diseases.

Given these potential reasons, clinical practitioners and researchers are urged to pursue further studies on Rg3. Currently, Rg3 is primarily administered orally. However, novel administration methods, such as catheter-based endocardial or intramyocardial injections, should be explored to enhance its

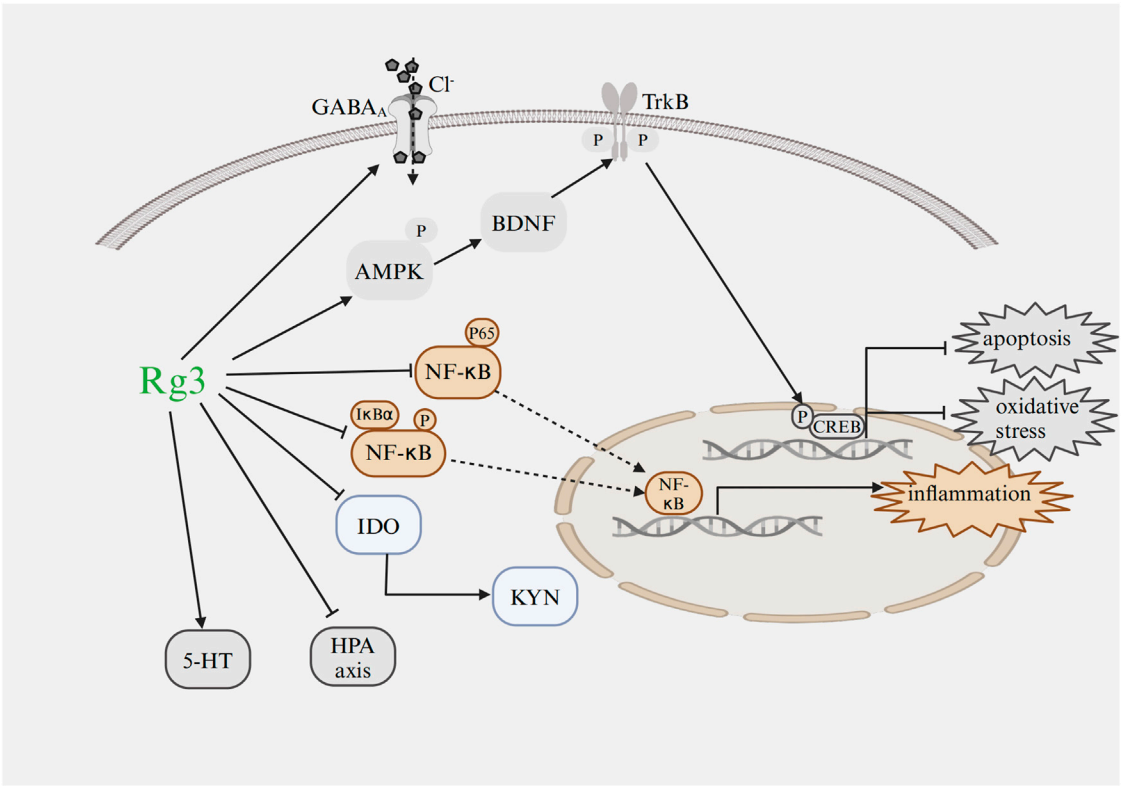

FIGURE 5
Mechanism of Rg3 on mental disorders Abbreviations: GABA, gamma aminobutyric acid; IDO, indoleamine 2,3-dioxygenase; NF-κB, nuclear factor κB; IκBα, inhibitor of kappa B alpha; KYN, kynurenine; HPA, hypothalamic-pituitary-adrenal; CREB, cyclic adenosine monophosphate response element binding protein; TrkB, tropomyosin-related kinase B; BDNF, brain-derived neurotrophic factor; 5-HT, serotonin; AMPK, AMP-activated protein kinase. Image created with BioRender.com, with permission.

clinical utility. Polyethylene glycol (PEG) is commonly utilized as a hydrophilic block in drug delivery systems for its resistance to protein adsorption and minimal toxicity (Li et al., 2020). Conversely, polypropylene sulfide (PPS) is favored as the hydrophobic block due to its pronounced hydrophobic properties. This PEG-b-PPS amphiphilic block copolymer demonstrates potential as a ROS-responsive nanovesicle, specifically designed for efficient drug delivery (Li et al., 2020). The combination of PEG and PPS offers a promising platform for enhancing the therapeutic effects of Rg3 (Li et al., 2020). Additionally, the development of Rg3-loaded nanoparticles with specific targeting abilities could enable non-invasive, tissue-specific drug delivery (Li et al., 2020). Furthermore, there is also a pressing need for extensive, well-controlled clinical trials to more accurately determine Rg3's benefits on cardiac and mental health. Regarding Rg3, there exist two variants: 20(R)-Rg3 and 20(S)-Rg3. The distinct impacts of these variants on cardiac and mental health remain to be elucidated. Given that these two epimers have already shown varied anticancer effects, their potential differential influence on heart diseases and mental disorders presents a research opportunity.

In summary, we have reviewed recent findings that illustrate the important role of Rg3 as a novel therapeutic agent in heart diseases and mental disorders. More clinical trials are motivated to be investigated for Rg3 in heart and mental diseases, especially in patients with the coexistence of cardiac diseases and psychiatric disorders.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

Rg3	Ginsenoside Rg3
CAD	Coronary artery disease
MDD	Major depressive disorder
MI	Myocardial infarction
HF	Heart failure
MIRI	Myocardial ischemia-reperfusion injury
SERCA2a	Sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase
ER	Endoplasmic reticulum
ROS	Reaction oxygen species
PDHc	Pyruvate dehydrogenase complex
DLD	Dihydrolipoamide dehydrogenase
ACY1	Aminoacylase-1
AngII	Angiotensin II
TGF-β1	Transforming growth factor-β1
AT1	Angiotensin type 1 receptor
CARP	Cardiac ankyrin repeat protein
ULK1	Unc51-like-kinase 1
FUNDC1	FUN14 domain-containing protein 1
PCI	Percutaneous coronary intervention
AMPK	AMP-activated protein kinase
ACC	Acetyl CoA carboxylase
TNF-α	Tumor necrosis factor-α
IL-1β	Interleukin 1 beta
NF-κB	Nuclear factor κB
eNOS	Endothelial nitric oxide synthase
Bcl-2	B cell lymphoma-2
Bax	Bcl2-associated X protein
H/R	Hypoxia/reoxygenation
PGC1-α	Peroxisome proliferators-activated receptor γ coactivator l alpha
IκBα	Inhibitor of kappa B alpha
DOX	Doxorubicin
SOD	Superoxide dismutase
Nrf	Nuclear factor erythroid 2-related factor
ARE	Antioxidant response element
PI3K	Phosphoinositide 3 kinase
PEG	Polyethylene glycol
PPS	Polypropylene sulfide
IRS	Insulin receptor substrate
TRP	Tryptophan
KYN	Kynurenine
BDNF	Brain-derived neurotrophic factor
CREB	Cyclic adenosine monophosphate response element binding protein
I_{GABA}	GABA-induced inward current
PTSD	Post-traumatic stress disorder
ADHD	Attention Deficit Hyperactivity Disorder
HPA	Hypothalamic-pituitary-adrenal
TrkB	Tropomyosin-related kinase B
SAM	Sympathetic-Adrenomedullary System
MDM4	Double minute 4 protein
CK	Creatine kinase
CK-MB	Creatine kinase-MB
LDH	Lactate dehydrogenase
PPAR-γ	Peroxisome proliferator-activated receptor γ
TGFBR1	Transforming Growth Factor Beta Receptor I
DCM	Diabetic cardiomyopathy
SIRT1	Sirtuin 1
GABA	Gamma aminobutyric acid
ISO	Isoproterenol
TIP60	Tat-interacting protein 60
Akt	Protein kinase B

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EDITED BY
Ismail Laher,
University of British Columbia, Canada

REVIEWED BY
Tao Ban,
Harbin Medical University, China
Xiaoyong Yu,
Shaanxi Provincial Hospital of Traditional Chinese Medicine, China

*CORRESPONDENCE
Wei Qin,
qinwei@mail.jnmc.edu.cn

†These authors have contributed equally to this work

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Non-coding RNAs: targets for Chinese herbal medicine in treating myocardial fibrosis

Minghui Wang[1,2†], Maocai Yan[2†], Liqiang Tan[3], Xiaona Zhao[2,4], Guoqing Liu[1,2], Zejin Zhang[2,5], Jing Zhang[2], Honggang Gao[2] and Wei Qin[2*]

[1]School of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan, Shandong, China, [2]School of Pharmacy, Jining Medical University, Rizhao, Shandong, China, [3]Department of Nasopharyngeal Carcinoma, Sun Yat-Sen University Cancer Center, Guangzhou, Guangdong, China, [4]School of Pharmacy, Weifang Medical University, Weifang, Shandong, China, [5]School of Pharmacy, Binzhou Medical University, Yantai, Shandong, China

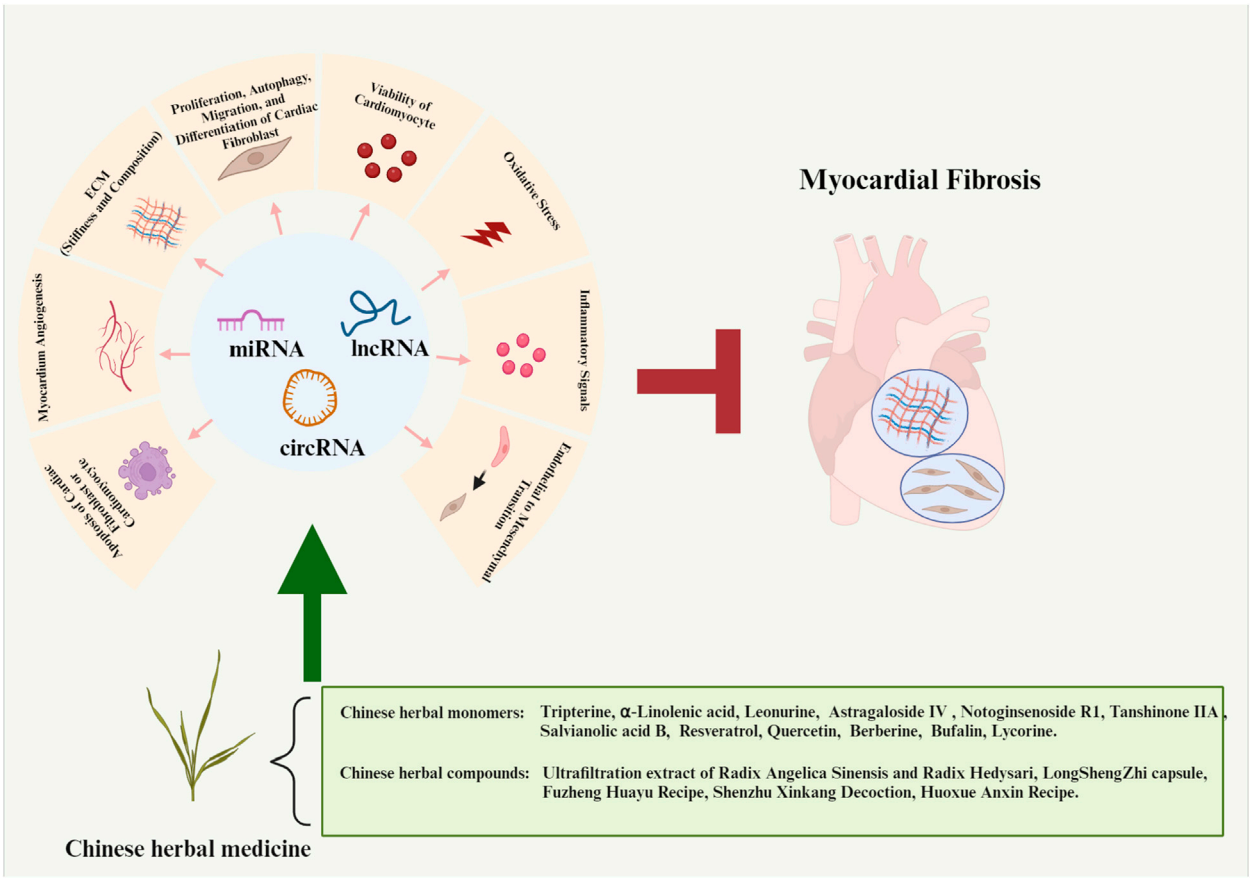
Cardiovascular diseases have become the leading cause of death in urban and rural areas. Myocardial fibrosis is a common pathological manifestation at the adaptive and repair stage of cardiovascular diseases, easily predisposing to cardiac death. Non-coding RNAs (ncRNAs), RNA molecules with no coding potential, can regulate gene expression in the occurrence and development of myocardial fibrosis. Recent studies have suggested that Chinese herbal medicine can relieve myocardial fibrosis through targeting various ncRNAs, mainly including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs). Thus, ncRNAs are novel drug targets for Chinese herbal medicine. Herein, we summarized the current understanding of ncRNAs in the pathogenesis of myocardial fibrosis, and highlighted the contribution of ncRNAs to the therapeutic effect of Chinese herbal medicine on myocardial fibrosis. Further, we discussed the future directions regarding the potential applications of ncRNA-based drug screening platform to screen drugs for myocardial fibrosis.

KEYWORDS

myocardial fibrosis, Chinese herbal medicine, ncRNAs, miRNAs, lncRNAs, circRNAs

1 Introduction

The main pathological features of myocardial fibrosis (MF) are excessive deposition of extracellular matrix in myocardial interstitium (Espeland et al., 2018). MF is a pathological manifestation of many cardiovascular diseases, like myocardial infarction (Talman and Ruskoaho, 2016), myocarditis (Tymińska et al., 2021), coronary heart disease (He et al., 2020) and hypertension (Rai et al., 2019). For example, following myocardial infarction, a large number of cardiac fibroblasts (CFs) are activated and transdifferentiate into myofibroblasts, which has stronger contraction function and extracellular matrix synthesis ability. Then increased collagen can deposit and form scar tissue in the infarcted area to provide support for the heart (González et al., 2018). Besides myocardial infarction, pressure overload due to hypertension or aortic stenosis (Verjans et al., 2018), volume overload induced by mitral valve (Dit Beaufils et al., 2021) or aortic valve insufficiency (Fragasso et al., 2022), and the release of inflammatory factors caused by oxidative stress (Lu et al., 2022) can also result in MF. MF is the end-stage pathological manifestation of most cardiovascular diseases and is closely related to cardiac death. It affects cardiac function in various ways. On one hand, perivascular fibrosis can impact



GRAPHICAL ABSTRACT

coronary blood supply, leading to myocardial ischemia, hypoxia, and even necrosis (Ytrehus et al., 2018). On the other hand, the deposition of a large number of collagen fibers results in an increase in ventricular wall hardness and a decrease in compliance, leading to reduced myocardial contraction, synchronization, and overall cardiac function (Sutton and Sharpe, 2000; Prabhu, 2005). Additionally, the deposition of collagen fibers in the matrix affects the conduction of myocardial electrical signals, easily forming reentrant rings and conduction blocks, which can result in arrhythmia (Francis Stuart et al., 2016). Therefore, studying the theraputic strategies of MF is helpful to slow down the progression of cardiovascular diseases and reduce the mortality rate of patients.

At present, the treatment of MF mainly involves two approaches: medication and surgery. Medication methods are more commonly used at the early stage of cardiovascular diseases to prevent the development of MF. These drug mainly include angiotensin-converting enzyme inhibitors (Francis Stuart et al., 2016), diuretics (Liu and Yu, 2022), β-blockers (Kobayashi et al., 2004), and anticoagulants (Oh et al., 2022). Surgical methods are used at the late stage of cardiovascular diseases, mainly including endocardial resection, atrioventricular valve repair/replacement, and heart transplantation. Endocardial resection can achieve the purpose of treatment by removing endocardial fibrous hyperplasia and calcification (Eckardt et al., 2000). Atrioventricular valve repair/replacement can repair/replace the fibrotic valve and restore its normal function (Mbanze et al., 2020). The heart transplantation can be performed when other treatments for heart problems have not worked, leading to heart failure (Srivastava and Kittleson, 2024). The aforementioned Western medicine methods offer the advantages of quick and potent effects, but their associated risks and side effects cannot be ignored. For instance, some medications may cause varying degrees of side effects, such as nausea, headaches, and liver function impairment; different patients may exhibit varied responses to the same treatment methods, leading to inconsistent treatment outcomes; some treatment methods may be relatively expensive, posing an economic burden on patients.

Chinese herbal medicine is an important historical treasure. Unlike Western medicine, Chinese herbal medicine offers the advantages of having minimal side effects in clinical applications. Prescriptions are tailored to individual patients, considering factors such as time and condition. From the perspectives of “blood stasis” and “phlegm turbidity”, Chinese herbal medicine is utilized in the treatment of MF, often in combination with compounds that promote blood circulation, remove blood stasis, and address phlegm and turbidity. Clinically, widely used formulations include Qishen Yiqi pill (Lv et al., 2021), Tongxinluo Capsule (Yin et al., 2019), Baoxin Decoction (Sun et al., 2017), Ginseng Dingzhi Decoction (Wanget al., 2022a), Huoxue Anxin Recipe (Wang et al., 2016), and others.

Non-coding RNAs (ncRNAs) are identified as RNA molecules with no coding potential (Yan and Bu, 2021). With the development of RNA sequencing technology, a large number of ncRNAs have

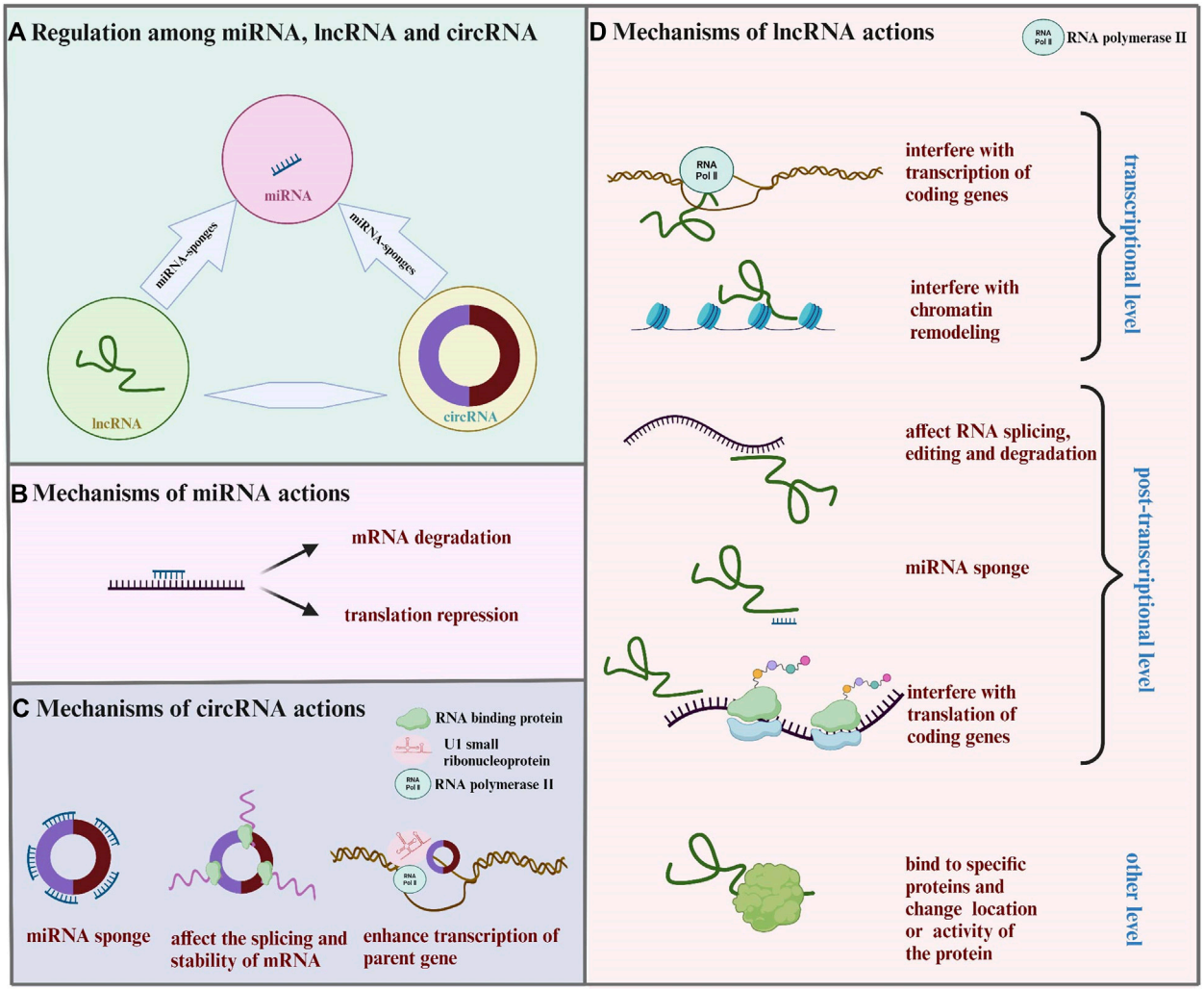


FIGURE 1
Mechanisms of controlling gene expression through ncRNAs. **(A)** LncRNA and circRNA act as miRNA sponges and affect the expression of miRNA and its target gene. **(B)** MiRNA binds to mRNA, leading to mRNA degradation or translation inhibition. **(C)** CirRNA combines with miRNA, RNA binding protein, U1 small ribonucleoprotein particles, and RNA polymerase II to affect the function of miRNA, interfere with the splicing or stability of mRNA, and enhance the transcription of parent genes. **(D)** LncRNA regulats gene expression from the transcriptional level, post-transcriptional level, and other level. Transcriptional level: lncRNA interferes with the transcription or chromatin remodeling of coding genes. Post-transcriptional level: lncRNA acts as miRNA sponges; lncRNA forms RNA double strand with mRNA, affecting mRNA splicing, editing, degradation, and translation. Other level: lncRNA binds to specific proteins, changing the location or activity of the protein.

been identified in different species and tissues. For a long time, it is generally believed that most genetic information is processed by protein-coding genes, while ncRNAs were regarded as junk nucleic acid sequences (Palazzo and Lee, 2015). In recent years, systematic analysis of cardiovascular genome and transcriptome has profoundly changed people's understanding of ncRNAs. Numerous studies have confirmed that ncRNAs are important regulatory factors in heart development and have an inseparable relationship with the occurrence and development of most cardiovascular diseases (Qu et al., 2016). By forming complexes with RNA, DNA, or proteins, ncRNAs modulate numerous pivotal signaling pathways implicated in MF (Chen et al., 2018b; Dilmaghnai et al., 2021). Moreover, the expression changes of ncRNAs in plasma and tissues can be regarded as biomarkers for early warning and predicting prognosis of MF (Jiang et al., 2020; Ghafouri-Fard et al., 2021). As such, they represent promising future clinical targets for modulating both MF and its associated cardiovascular conditions. In recent years, studies have shown that ncRNAs are the targets of Chinese herbal medicine in the treatment of MF. In this study, we have discussed recent progress in the modulation of ncRNAs through Chinese herbal medicine for managing MF, with a focus on herbal monomers and compounds.

2 Mechanisms of ncRNAs in regulating gene expression

In the past few decades, ncRNAs have been proved to regulate gene expression at multiple levels in the occurrence and development of MF, and act as new drug targets for MF

treatment. These ncRNAs mainly include microRNA (miRNA), long non-coding RNA (lncRNA), and circular RNA (circRNA), of which miRNA-mediated regulation have mostly been studied and documented (Dong et al., 2019b). Intrestingly, there is also an interaction between the three ncRNAs, called competitive endogenous RNA (ceRNA) mechanism, which complicates gene regulation. The mechanisms of ncRNAs in regulating gene expression and their inter-regulations are shown in Figure 1.

2.1 MiRNA in regulating gene expression

MiRNA is a highly conserved gene family with a length of about 22–25 nucleotides (Davoodvandi et al., 2021). MiRNA binds to the untranslated region at the 3' end of mRNA to inhibit mRNA transcription or translation (Mohr and Mott, 2015). Studies have shown that pri-miRNA, the primary transcription product of miRNA, is cut into hairpin precursor miRNA (pre-miRNA) by Drosha enzyme of ribonuclease 3 family. After preliminary cutting, pre-miRNA is transported from the nucleus to the cytoplasm by transporter. Then, the pre-miRNA is further cut by the combined action of Dicer enzyme of ribonuclease 3 family and dsRNA binding protein (dsRBP). Finally, the mature miRNA binds to argonaute (AGO) protein to form RNA-inducing silencing complex (RISC), leading to the interaction between the complex and the target mRNA (Leitão and Enguita, 2022). There are two ways in which miRNA regulates gene expression: when miRNA is completely complementary to mRNA, the mRNA can be directly cut; when miRNA binds with mRNA incompletely, the translation of mRNA is prevented but the stability of mRNA is not affected (Fabian et al., 2010).

Through regulating fibrosis-related factors, miRNA plays various roles in MF. For example, mir-338-3p acts as a therapeutic target in MF through fibroblast growth factor receptor 2 (FGFR2) suppression (Huang et al., 2022a). MiR-125b is critical for induction of MF by targeting p53 and Apelin mRNA (Nagpal et al., 2016). The matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) play crucial roles as regulators of extracellular matrix turnover and tissue remodeling, significantly influencing MF (Serraino et al., 2023). A previous study has shown that miR-146b-5p can bind to TIMP4 mRNA, regulating the balance between TIMP4 and MMP9, which is associated with atrial fibrosis (Ye et al., 2021). Additionally, MMP2 and MMP9 have been identified as potential targets for miR-29a and miR-133a (Jones et al., 2011). Connective tissue growth factor (CTGF) exerts chemotactic and mitogenic effects on fibroblasts, closely related to the occurrence and development of fibrosis in various tissues and organs (Ramazani et al., 2018). MiR-30a attenuates MF in rats by targeting CTGF (Chen et al., 2018a). Furthermore, Galectin-3 promotes the proliferation and collagen synthesis of CFs, while miR-335 inhibits MF by directly targeting this gene Sun.

2.2 LncRNA in regulating gene expression

Compared with miRNA, the length of lncRNA is usually longer, generally more than 200 nt, which has the similar structure with mRNA (Jha et al., 2023). Firstly, ceRNA mechanism is one of the important ways for lncRNA to regulate genes (Huang, 2018). As a natural miRNA sponge, lncRNA competes with mRNA to bind with miRNA, which affects gene silencing induced by miRNA. For example, lncRNA PFL acts as a ceRNA of let-7d to promote fibrogenesis (Liang et al., 2018), Besides ceRNA mechanism, lncRNA can also directly bind to DNA, mRNA and protein. This modulation can be categorized into three levels: transcription level, post-transcription level and other level (Li et al., 2023b). Transcriptional level means lncRNA can interfere with the transcription or chromatin remodeling of coding genes (Dykes and Emanueli, 2017). For example, lncTCF7 recruit switch/sucrose non-fermentable (SWI/SNF) complex to transcription factor 7 (TCF7) promoter region, leading to transcription of TCF7 gene (Wang et al., 2015b). Post-transcriptional level means lncRNA can influence mRNA splicing or translation (Ma et al., 2013). For example, lncRNA Safe can complementarily combine with secreted frizzled-related protein 2 (Sfrp2) mRNA to form a Safe-Sfrp2 RNA duplex to stabilize each other (Hao et al., 2019). Moreover, lncRNA can also bind to specific proteins, changing the location of the protein or regulating its activity (Guttman and Rinn, 2012). For example, lncRNA HOX transcript antisense RNA (HOTAIR) could bind with polypyrimidine tract-binding protein 1 (PTBP1) to increase the stability of Wnt5a (Tan et al., 2022).

The mechanism of regulating MF by lncRNA is complicated, with numerous lncRNAs involved in the ceRNA network to exert their functions. For example, lncRNA DANCR targets miR-758-3p to regulate proteoglycan 4 (PRG4) and the downstream Smad pathway, influencing the progression of cardiac dysfunction and fibrosis (Huang and Huang, 2023). LncRNA CFAR promotes MF via targeting miR-449a-5p to regulate the lysyl oxidase-like protein-3 (LOXL3)/mammalian target of rapamycin (mTOR) axis (Zhang et al., 2023). LncRNA TUG1 exacerbates MF in diabetic cardiomyopathy by modulating the miR-145a-5p/cofilin-2 (Cfl2) axis (Wang et al., 2023). Unlike the above mechanism, some lncRNAs regulate MF by directly decoying proteins. For example, the regulatory role of lncRNA Wisper in CFs proliferation, migration, and survival depends on its association with TIA1-related protein (Micheletti et al., 2017). LncRNA MetBil directly binds to methyltransferase like 3 (METTL3) protien to regulate its expression in ubiquitin-proteasome pathway, thereby regulating the expression of the methylated fibrosis-associated genes in ischemia-induced MF (Zhuang et al., 2023).

2.3 CircRNA in regulating gene expression

CircRNA has a closed loop structure without 5′cap and 3′polyA tail, and the average length of circRNA in human body is about 500 nt (Dragomir and Calin, 2018). CircRNA contains abundant miRNA binding sites and can be used as a sponge of miRNA (Salmena et al., 2011). For example, circRNA-005647 has a binding site with miR-27b-3p and inhibits the binding of miR-27b-3p with fibrosis-related genes (Yuan et al., 2019). In addition to ceRNA mechanism, circRNA can affect the splicing and stability of mRNA by binding to RNA binding protein (RBP). For example, circFndc3b enhances the expression and signal transduction of vascular endothelial growth factor-A (VEGF-A) by interacting

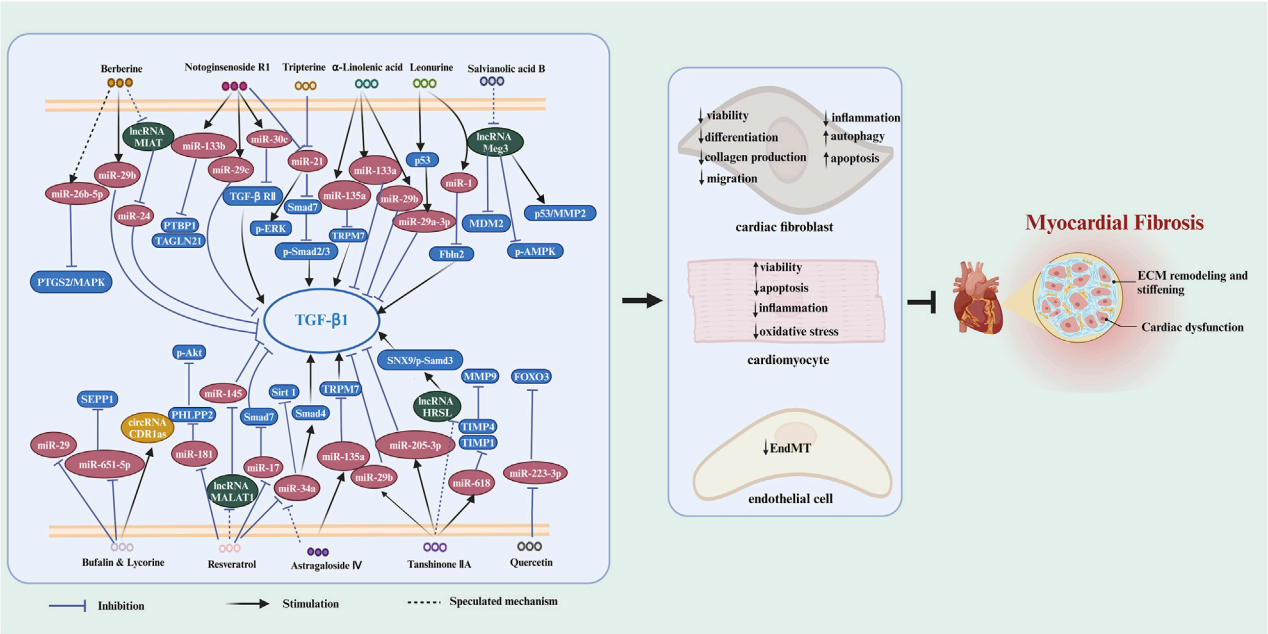


FIGURE 2
The signaling pathways of Chinese herbal monomers in treating MF by interfering with ncRNAs. Chinese herbal monomers regulate ncRNAs to inhibit the viability, differentiation, migration, inflammation, and collagen production of cardiac fibroblasts while triggering apoptosis and autophagy in these cells. Furthermore, these herbal monomers can also inhibit inflammation, oxidative stress, and apoptosis in cardiomyocytes and enhance their viability through ncRNA regulation. Additionally, EndMT can also be inhibited by Chinese herbal monomers in a ncRNAs-dependent way. These mechanisms collectively contribute to the ultimate inhibition of myocardial fibrosis. The blank arrow indicates promotion, the blue T-shaped arrow indicates inhibition, and the black dotted arrow indicates the presumed regulatory function.

with RBP fused in sarcoma (FUS) (Garikipati et al., 2019). What's more, circRNA can also bind to the promoter region and enhance the transcription of its parent gene by interacting with U1 small ribonucleoprotein particles and RNA polymerase II (Lun et al., 2023).

The mechanisms currently reported for circRNA in regulating MF mainly involve acting as a ceRNA, interacting with proteins, and encoding proteins. For example, circSMAD4 promotes MF by acting as a sponge against miR-671-5p (Jeong et al., 2023). CircYap directly binds to tropomyosin-4 (TMP4) and gamma-actin (ACTG) to make the interaction between the two proteins more stable, resulting in the inhibition of actin polymerization and subsequent MF (Wu et al., 2021). Circ_0036176 has the ability to encode a protein containing 208 amino acids named Myo9a-208, which mediates the inhibitory effect of circ_0036176 on the proliferation of CFs (Guo et al., 2022).

3 Chinese herbal medicine relieves MF through regulating ncRNAs

Chinese herbal medicine has the characteristics of wide sources and small side effects, thus having a unique advantage in treating human diseases including MF. Recent studies have suggested that Chinese herbal medicine can exert anti-MF effects by regulating ncRNAs. 12 kinds of Chinese herbal monomers and 5 kinds of Chinese herbal compounds have been shown to treat MF by interfering with ncRNAs. The herbal monomers include tripterine, α-linolenic acid, leonurine, astragaloside IV, notoginsenoside R1, tanshinone IIA, salvianolic acid B, resveratrol, quercetin, berberine, bufalin, and lycorine. The pharmacological action and mechanisms of these Chinese herbal monomers are summarized below in Figure 2. The chemical structural formulas of Chinese herbal monomers are shown in Figure 3. The Chinese herbal compounds include trafiltration extract of radix angelica sinensis and radix hedysari, Longshengzhi capsule, Fuzheng Huayu Recipe, Shenzhu Xinkang Decoction, and Huoxue Anxin Recipe. The pharmacological action and mechanisms of these Chinese herbal compounds are summarized below in Figure 4.

3.1 Chinese herbal monomers and their targeting ncRNAs in treating MF

3.1.1 Tripterine

Chinese herb *Tripterygium wilfordii* Hook. F. has the effects of dispelling dampness, relieving swelling and pain, and resisting inflammation (Zeng et al., 2024). Triptolide and tripterine are the two most active components in the extract of *Tripterygium wilfordii* Hook. F. In recent years, it has been found that tripterine can inhibit CFs viability and collagen production by down-regulating the expression of miR-21, the activator of ERK signaling pathway, leading to relieved MF and cardiac dysfunction (Cheng et al., 2016). In fact, miR-21 has been found to be a pro-fibrotic factor in various animal models, targeting multiple fibrotic pathways and promoting MF. For example, in a mouse model of myocardial

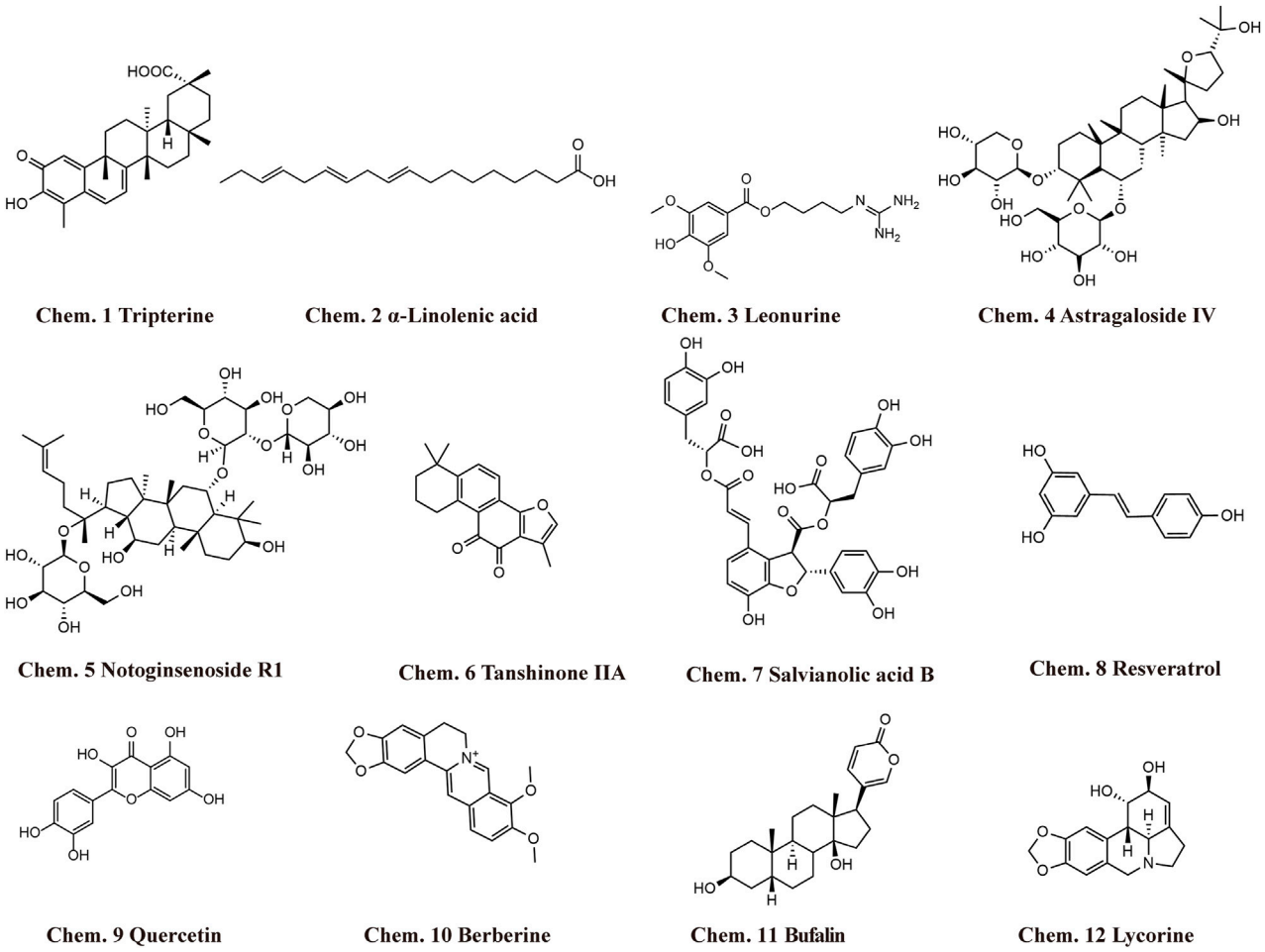


FIGURE 3
Structural formula of Chinese herbal monomers that exhibit anti-MF activity by interfering with ncRNAs.

infarction, miR-21 promotes CF activation and MF via TGF-β/Smad7 signaling pathway (Yuan et al., 2017). In another diabetes-induced MF mouse model, silencing miR-21 inhibits high glucose-induced endothelial to mesenchymal transition (EndMT) (Li et al., 2020). These results suggest that the anti-fibrotic effect of tripterine is mediated by miR-21 and the downstream ERK, TGF-β, and EndMT signaling pathways.

3.1.2 α-Linolenic acid (ALA)

Flaxseed oil is extracted from *Linum usitatissimum* L., and is rich in omega-3 fatty acids such as ALA. It is usually used to lower cholesterol, resist atherosclerosis, and reduce heart load (Prasad et al., 2020). A study has shown that in a rat model of myocardial infarction, flaxseed oil exerts cardioprotective effect and decreases collagen deposition via selectively up-regulaing the expression of miR-133a, miR-135a, and miR-29b. The author speculated this effect may be attributed to ALA component in the flaxseed oil (Parikh et al., 2020). Previous studies have shown that the three miRNAs are all anti-fibrotic miRNAs. MiR-133a reduces MF by suppressing transforming growth factor-β1 (TGF-β1) signaling in an acute myocardial infarction model (Yu et al., 2019). MiR-135a could target transient receptor potential melastatin 7 (TRPM7) to inhibit the activation of TGF-β/Smads pathway, thus relieving MF (Wei et al., 2020). MiR-29b inhibits many genes involved in extracellular matrix formation and fibrosis, such as Col1a1, Col1a2, Col3a1, fibrillin 1, Elastin, and TGF-β1 (Rooij et al., 2008; Zhang et al., 2014).

3.1.3 Leonurine

Leonurine, an alkaloid extracted from *leonurus japonicus* Houtt., has been shown to have various pharmacological effects including protecting myocardial ischemia-reperfusion injury, resisting blood platelet aggregation, reducing blood viscosity, promoting angiogenesis, lowering blood pressure, and inducing diuresis (Huang et al., 2021). Recent studies have shown that leonurine can treat MF induced by isoproterenol by upregulating miR-1, which could directly target Fibullin-2 (Fbln2) to reverse cardiac remodeling (Karakikes et al., 2013; Lu et al., 2018). In addition, evidence has shown that all mature miR-29 family members in post-myocardial infarction tissues are downregulated (Wang et al., 2021). Leonurine treatment can significantly upregulate the expression of miR-29a-3p and downregulate its target proteins including TGF-β, Col3a1, and Col1a1, to attenuate fibrosis and cardiac remodeling (Wang et al.,

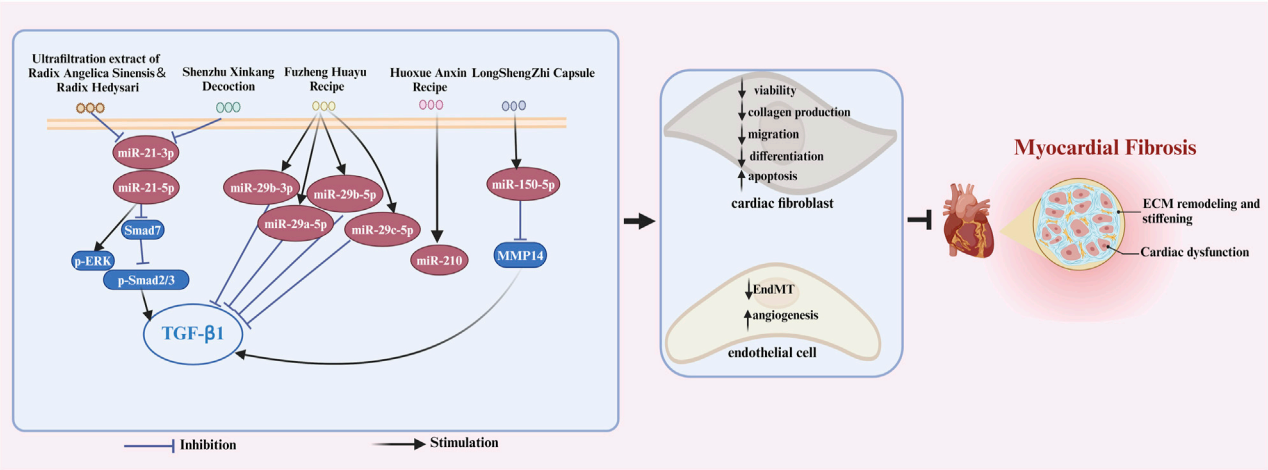


FIGURE 4
The signaling pathways of Chinese herbal compounds in treating MF by interfering with ncRNAs. Chinese herbal compounds regulate ncRNAs to inhibit the viability, differentiation, migration, and collagen production of cardiac fibroblasts while triggering apoptosis in these cells. Moreover, these herbal compounds can also inhibit EndMT and promote myocardium angiogenesis through ncRNAs regulation. These mechanisms collectively contribute to the ultimate inhibition of myocardial fibrosis. The blank arrow indicates promotion, the blue T-shaped arrow indicates inhibition.

2021). What's more, a study confirmed that leonurine can also promote apoptosis of CFs through regualting miR-29a-3p (Xi et al., 2023). These evidences suggest that the anti-fibrotic effect of leonurine may be mediated by miR-1 and miR-29a-3p.

3.1.4 Astragaloside IV

Astragaloside IV is one of the best bioactive components from the root of *Astragalus membranaceus* (Fisch.) Bunge (Jing et al., 2021). Studies have shown that astragaloside IV inhibits MF by up-regulating the expression of miR-135a which targets transient receptor potential melastatin 7 (TRPM7) (Wei et al., 2020). Moreover, in an experiment of high glucose-induced injury of cardiomyocytes, it was found that astragaloside IV could reduce the expression of miR-34a (Zhu et al., 2019). According to previous studies, inhibition of miR-34a can treat MF by inhibiting cardiomyocytes apoptosis (Dong F. et al., 2019a). Therefore, it can be speculated that astragaloside IV may play an anti-fibrotic role through repressing cardiomyocytes apoptosis by decreasing miR-34a.

3.1.5 Notoginsenoside R1

Panax notoginseng saponins are the main component of the roots of *Panax notoginseng* (Burk.) F. H. Chen. Panax notoginseng saponins have the effects of promoting blood circulation, removing blood stasis, and dredging collaterals, which are commonly used to treat coronary heart disease and blood stasis syndrome (Jiao et al., 2021; Yang et al., 2022). Notoginsenoside R1 is the primary active component of Panax notoginseng saponins. It was found that notoginsenoside R1 inhibits isoproterenol-induced MF through the intervention of miRNA-mRNA regulatory network, among which the expression level of miR-21 decreased, while miR-29c, miR-30c and miR-133b increased (Ning, 2016). These miRNAs have all been proven to be fibrosis-related miRNAs. It has been shown that miR-21 activates ERK signaling pathway, EndMT and TGF-β signaling pathway to promots MF (Cheng et al., 2016; Yuan et al., 2017; Li et al., 2020). MiR-29c has been proved to target multiple fibroisis-related genes including Col1a1, Col1a2, Col3a1 and Col5a1, fibrllin 1 and TGF-β1 (Liu et al., 2017) MiR-30c inhibits the proliferation, differentiation, migration and collagen production of CFs by targeting TGF-βRII (Xu et al., 2018); MiR-133b has also been reported to alleviate doxorubicin-induced cardiomyocyte apoptosis and MF by targeting PTBP1 and transgelin 2 (TAGLN2) (Li et al., 2021). Therefore, notoginsenoside R1 can target multiple fibrosis-related miRNA and can be regarded as attractive anti-fibrotic candidate medicine.

3.1.6 Tanshinone IIA

Tanshinone IIA is extracted from the dried root and rhizome of *Salvia miltiorrhiza* Bunge, which exerts a wide range of cardioprotective effects in the diseases like myocardial infarction, myocardial ischemia-reperfusion injury, myocardial hypertrophy, atherosclerosis, and cardiomyopathy (Zhang et al., 2019; Yang et al., 2020). Recent studies have shown that tanshinone IIA can relieve MF through up-regulating miR-29b (Yang et al., 2015), miR-205-3p (Qiao et al., 2021), and miR-618 (Yan et al., 2022) expressions. MiR-29b and miR-205-3p could downregulate the expression of TGF-β1, Col1a1, and Col3a1, so as to resist MF following myocardial infarction (Yang et al., 2015; Qiao et al., 2021). MiR-618 could inhibit the expression of TIMP1 and TIMP4 to mediate the anti-fobrotic effects of tanshinone IIA on CFs (Yan et al., 2022). In addition, it has been found that tanshinone IIA can inhibit fibroblast proliferation by down-regulating lncRNA human-specific regulatory loci (HSRL) in skin hypertrophic scar tissue. HSRL could promote the expression of sorting connexin 9 (SNX9) and strengthen its interaction with p-Smad3, thus activating TGF-β signaling (Shi et al., 2020). Therefore, inhibiting HSRL may also mediate the anti-fibrotic effect of tanshinone IIA.

3.1.7 Salvianolic acid B

Salvianolic acid B is also derived from the root and rhizome of *Salvia miltiorrhiza* Bunge. A study showed that lncRNA maternally expressed gene 3 (Meg3), mainly expressed in CFs, is a new promotor of MF. Silencing Meg3 prevents MMP2 production, leading to the decreased MF and improved cardiac function (Piccoli et al., 2017). In cardiomyocytes of oxygen and glucose deprivation (OGD), salvianolic acid B was reported to represses Meg3 expression, which influences murine double minute 2 (MDM2)/p53 and AMP-activated Protein Kinase (AMPK) signalling pathways, leading to incresed viability and reduced apoptosis of cardiomyocytes (Yang et al., 2019a). Therefore, it is speculated that salvianolic acid B may have the cardioprotective effect of inhibiting MF by down-regulating Meg3, but more researches are needed to test this hypothesis.

3.1.8 Resveratrol

Resveratrol is an active polyphenol, derived from many herbal medicines, such as *Morus alba* L., *Polygonum cuspidatum* Sieb. et Zucc., and *Rubus idaeus* L. Resveratrol is proved to have anti-bacterial, anti-inflammatory and immunomodulatory effects (Malaguarnera, 2019). In cardiovascular system, it exerts protective effects on atherosclerosis, myocardial infarction, and heart failure (Raj et al., 2021). Recent studies showed that resveratrol can inhibit proliferation and induce cell death of CFs (Lieben Louis et al., 2019). A study assessed the impact of resveratrol on microRNAs linked to MF, and found resveratrol inhibits the expressions of miR-17, miR-34a and miR-181a in TGF-β1-induced CFs (Zhang et al., 2018). Overexpression of miR-17 decreases Smad7 expression level, indirectly promotes TGF-β1 signaling (Zhang et al., 2018). MiR-34a activates TGF-β1 signaling through increasing Smad4 expression (Huang et al., 2014). MiR-181a suppresses the expression of PH domain leucine-rich repeat protein phosphatase 2 (PHLPP2) and subsequently activates AKT signaling, leading to enhanced proliferation of keloid fibroblast cells (Rang et al., 2016). In addition, resveratrol was reported to inhibit the expression of lncRNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1), which could act as a ceRNA of miR-145 and promote MF progression (Yang et al., 2019b; Huang et al., 2019). Therefore, downregulation of MALAT1 may be one of the potential mechanisms of resveratrol in treating MF. It is evident that resveratrol, with its multiple ncRNA targets, holds significant promise for the management of MF.

3.1.9 Quercetin

Quercetin is a kind of flavonol that widely exists in flowers, leaves and fruits of many plants, such as *Sophora japonica* L, *Asparagus officinalis* L., and *Acanthopanax senticosus* (Rupr.et Maxim.) Harms. Previous studies have shown that quercetin restrains the level of fibrotic proteins including TGF-β1, α-SMA, Col1a1, and Col3a1 in heart tissue of myocardial infarction model (Albadrani et al., 2021). Studies are progressively uncovering that the promotion of CFs autophagy can effectively inhibit MF and enhance cardiac function (Wang et al., 2015a). Quercetin was found to prevent isoprenaline-induced MF by increasing autophagy of CFs via decreasing miR-223-3p and increasing forkhead Box O3 (FOXO3) (Hu et al., 2021).

3.1.10 Berberine

Berberine is a quaternary ammonium alkaloid contained in the rhizome of *Coptis chinensis* Franch. and has various cardiovascular protective effects, such as anti-heart failure, anti-arrhythmia, and lowering cholesterol effects (Pagliaro et al., 2015; Zhao et al., 2020). It was found that berberine relieves hypertension-induced MF by increasing the expression of miR-29b and decreasing its targets Col1a1 and Col3a1 (Zheng et al., 2020). Moreover, in a myocardial ischemia-reperfusion mouse model, the protective effect of berberine is exerted by inducing miR-26b-5p and inhibiting its downstream PTGS2 and MAPK members, which results in the increased viability, and decresed apoptosis, inflammatory, and oxidative stress in cardiomyocytes (Jia et al., 2022). Since the injury of the cardiomyocytes is the major causes of MF, it is speculated that miR-26b-5p may be a potential target of berberine in the treatment of MF. In addition, berberine was also proved to inhibit lncRNA myocardial infarction-associated transcript (MIAT) to improve myocardial hypertrophy (Zeng et al., 2019). Since MIAT is a pro-fibrotic lncRNA governing MF by down-regulating miR-24 and up-regulating Furin and TGF-β1 (Qu et al., 2017), we can infer that berberine may have the effect to improve MF by inhibiting MIAT.

3.1.11 Bufalin and lycorine

Bufalin comes from dried *toad*, while lycorine is an alkaloid found in the bulb of *Lycoris radiata* (L'Hér.) Herb. Recently, high-throughput natural compound library screening identified bufalin and lycorine to be effective anti-fibrotic molecules in hypertension-induced MF mouse model (Schimmel et al., 2020). The study found the level of miR-671-5p is reduced after treatment of CFs with bufalin and lycorine, which leads to the increased expression of anti-fibrotic protein selenoprotein P1 (SEPP1) (Schimmel et al., 2020). Another study discovered that bufalin and lycorine can reduce the expression of miR-29 while increasing the expression of circRNA CDR1as. This, in turn, leads to a decrease in the infarction area and fibrotic area in a heart failure pig model (Mester-Tonczar et al., 2020). Consequently, the anti-fibrotic impact of bufalin and lycorine can be ascribed to the reduction of miR-671-5p and miR-29 levels and the elevation of CDR1as levels.

3.2 Chinese herbal compounds and their targeting ncRNAs in treating MF

3.2.1 Ultrafiltration extract of radix angelica sinensis and radix hedysari

Radix Angelica Sinensis is usually used in combination with other drugs to treat cardiovascular diseases such as radiation-induced heart disease, atherosclerosis, and ischemic heart disease (Huang et al., 2022b; Li et al., 2023a; Yuan et al., 2023), while Radix Hedysari has been proved to have significant effects in treating non-alcoholic fatty liver disease (Sun et al., 2014). In a rat model of X-irradiation-induced MF, ultrafiltration extract derived from dried root of Radix Angelica Sinensis and Radix Hedysari downregulates miR-21-3p and miR-21-5p, inducing the apoptosis of CFs and alleviating MF (Ma et al., 2019). It has been shown that miR-21 activates ERK signaling pathway, EndMT, and TGF-β signaling pathway to promote MF (Cheng et al., 2016;

TABLE 1 Mechanisms of Chinese herbal medicine in treating MF by regulating ncRNAs.

Herbal medicine	ncRNAs	Direct ncRNAs' targets	Biological function	Refrences
Tripterine	miR-21↓	Smad7	↓CFs viability, differentiation, migration, and collagen production	Cheng et al. (2016), Yuan et al. (2017), Li et al. (2020)
			↓EndMT process	
α-Linolenic acid	miR-29b↑	TGF-β1	↓CFs differentiation and collagen production	Rooij et al. (2008), Zhang et al. (2014), Parikh et al. (2020)
		Col1a1		
		Col1a2		
		Col3a1 fibrillin 1		
		Elastin		
	miR-133a↑	?	↓CFs differentiation and collagen production	Yu et al. (2019), Parikh et al. (2020)
	miR-135a↑	TRPM7	↓CFs viability, differentiation, and collagen production	Parikh et al. (2020), Wei et al. (2020)
Leonurine	miR-1↑	Fbln2	↓CFs collagen production	Karakikes et al. (2013), Lu et al. (2018)
	miR-29a-3p↑	TGF-β1	↓CFs viability, differentiation, migration, and collagen production	Wang et al. (2021), Xi et al. (2023)
		Col3a1	↑CFs apoptosis	
		Col1a1		
Astragaloside IV	miR-135a↑	TRPM7	↓ CFs viability, differentiation, and collagen production	Wei et al. (2020)
	*miR-34a↓	Sirt1	↓CMs apoptosis	Dong et al. (2019a), Zhu et al. (2019)
Notoginsenoside R1	miR-21↓	Smad7	↓CFs viability, differentiation, migration, and collagen production	Cheng et al. (2016); Ning (2016), Yuan et al. (2017), Li et al. (2020)
			↓EndMT process	
	miR-29c↑	?	↓CFs differentiation and collagen production	Ning (2016); Liu et al. (2017)
	miR-30c↑	TGF-β RII	↓CFs viability, differentiation, migration, and collagen production	Ning (2016); Xu et al. (2018)
	miR-133b↑	PTBP1	↓CFs collagen production	Ning (2016); Li et al. (2021)
		TAGLN2	↓CMs apoptosis	
Tanshinone IIA	miR-29b↑	?	↓CFs differentiation and collagen production	Yang et al. (2015)
	miR-205-3p↑	TGF-β1	↓CFs collagen production	Qiao et al. (2021)
	miR-618↑	TIMP1	↓CFs viability, differentiation, and collagen production	Yan et al. (2022)
		TIMP4		
	*lncRNA HSRL↓	SNX9	↓CFs viability, differentiation, and collagen production	Shi et al. (2020)
Salvianolic acid B	*lncRNA Meg3↓	p53	↓CFs collagen production	Piccoli et al. (2017), Yang et al. (2019a)
			↓CMs apoptosis	
			↑CMs viability	
Resveratrol	miR-17↓	Smad7	↓CFs viability and collagen production	Zhang et al. (2018)
	miR-34↓	Smad4	↓ CFs viability, differentiation, migration, and collagen production	Huang et al. (2014), Zhang et al. (2018)
	miR-181a↓	PHLPP2	↓CFs viability	Rang et al. (2016), Zhang et al. (2018)

(Continued on following page)

TABLE 1 (*Continued*) Mechanisms of Chinese herbal medicine in treating MF by regulating ncRNAs.

Herbal medicine	ncRNAs	Direct ncRNAs' targets	Biological function	Refrences
	*lncRNA MALAT1↓	miR-145	↓CFs viability, differentiation, and collagen production	Yang et al. (2019b), Huang et al. (2019)
Quercetin	miR-223-3p↓	FOXO3	↑CFs autophagy	Hu et al. (2021)
			↓CFs viability and collagen production	
Berberine	*miR-26b-5p↑	?	↑CMs viability	Jia et al. (2022)
			↓CMs apoptosis, inflammation, and oxidative stress	
	miR-29b↑	?	↓CFs collagen production	Zheng et al. (2020)
	*lncRNA MIAT↓	miR-24	↓CFs viability and collagen production	Qu et al. (2017), Zeng et al. (2019)
Bufalin and Lycorine	miR-29↓	?	↓CFs collagen production	Mester-Tonczar et al. (2020)
	circRNA CRD1as↑	?		
	miR-671-5p↓	SEPP1	↓CFs differentiation, collagen production, and inflammation	Schimmel et al. (2020)
Ultrafiltration extract of Radix Angelica Sinensis and Radix Hedysari	miR-21-3p↓	Smad7	↓CFs viability, differentiation, migration, and collagen production	Cheng et al. (2016), Yuan et al. (2017), Ma et al. (2019), Li et al. (2020)
	miR-21-5p↓		↑CFs apoptosis	
			↓EndMT process	
LongShengzhi capsule	miR-150-5p↑	MMP14	↓CFs collagen production	Gu et al. (2022)
Fuzheng Huayu Recipe	miR-29b-3p↑	?	↓CFs collagen production	Qi et al. (2019)
	miR-29a-5p↑			
	miR-29b-5p↑			
	miR-29c-5p↑			
Shenzhu Xinkang Decoction	miR-21↓	?	↓EndMT process	Zhai et al. (2022)
Huoxue Anxin Recipe	miR-210↑	?	↑Myocardium angiogenesis	Wang et al. (2016)

(Marked with * is the speculated mechanism).

Abbreviations: CMs, cardiomyocytes; CFs, cardiac fibroblasts; circRNA, circular RNA; EndMT, endothelial to mesenchymal transition; Fbln2, Fibullin-2; FOXO3, forkhead box O3; lncRNA, long noncoding RNA; MALAT1, metastasis associated lung adenocarcinoma transcript 1; Meg3, maternally expressed gene 3; MF, myocardial fibrosis; miRNA, microRNA; MIAT, myocardial infarction-associated transcript; MMP14, matrix metalloproteinase 14; PHLPP2, PH, domain leucine-rich repeat protein phosphatase 2; PTBP1, polypyrimidine tract-binding protein 1; SEPP1, selenoprotein P1; SNX9, sorting connexin 9; TAGLN2, transgelin 2; TIMP1, tissue inhibitors of matrix metalloproteinase 1; TIMP4, tissue inhibitors of matrix metalloproteinase 4; TRPM7, transient receptor potential melastatin 7.

Yuan et al., 2017; Li et al., 2020). Therefore, the Radix Angelica Sinensis and Radix Hedysari ultrafiltration extract may be developed as a medical countermeasure for the mitigation of radiation-induced MF.

3.2.2 LongShengZhi capsule

Buyang Huanwu Decoction is a famous herbal prescription that has been used to treat stroke for centuries (Gao et al., 2021). Previous studies have shown that Buyang Huanwu Decoction can alleviate MF (Wang et al., 2022b). The compatible components of LongShengZhi capsule are similar to those of Buyang Huanwu Decoction, and this is referred to as the modern application of Buyang Huanwu Decoction. A study found that LongShengZhi capsule attenuates Angiotensin II-induced cardiac hypertrophy and fibrosis in rats. Mechanically, Longshengzhi capsule up-regulats miR-150-5p to target MMP14 in CFs, leading to reduced cardiac remodeling (Gu et al., 2022).

3.2.3 Fuzheng Huayu Recipe

Fuzheng Huayu Recipe, a traditional Chinese herbal prescription, is often used in China to treat fibrosis (Wang et al., 2010; Sun et al., 2022). Recently, a study has suggested that Fuzheng Huayu Capsule inhibits myocardial infarction-induced MF by facilitating the expression of miR-29b-3p, miR-29a-5p, miR-29b-5p, and miR-29c-5p (Qi et al., 2019). It has been shown that miR-29 family are all anti-fibrotic factors with the effect of inhibiting TGF-β1 signaling and its downstream targets, resulting in reduced proliferation and collagen production of CFs (Rooij et al., 2008; Zhu et al., 2013; Zhang

et al., 2014). Therefore, miR-29 family are the key mediators for the anti-fibrotic effect of Fuzheng Huayu Recipe.

3.2.4 Shenzhu Xinkang Decoction

Shenzhu Xinkang Decoction is a representative prescription for the treatment of chronic heart failure and fibrosis (Zhao et al., 2023). EndMT has been shown to contribute to cardiac fibrosis (Zeisberg et al., 2007) and Shenzhu Xinkang Decoction was proved to inhibit EndMT to play an anti-fibrotic role. The possible mechanism is related to the downregulation of miR-21 level and inhibition of PTEN/PI3K/AKT pathway (Zhai et al., 2022).

3.2.5 Huoxue Anxin Recipe

Huoxue Anxin Recipe is a novel formula of Chinese herbal medicine that has a good cardioprotective effect, such as promoting myocardial angiogenesis, exhibiting anti-oxidative stresses activity, and improving cardiac function during myocardial infarction (Zhang et al., 2012; Zhang et al., 2013; Wang et al., 2016). A recent study has demonstrated that Huoxue Anxin Recipe could reduce the infarction area, alleviate fibrosis, and improve the cardiac function of myocardial infarction rats, which is mainly attributed to enhanced angiogenesis by upregulation of miR-210 and VEGF (Wang et al., 2016).

4 Conclusion and perspective

The targeted relationship between Chinese herbal medicine and ncRNAs are hot spots in current research, which opens up a new avenue for exploring the mechanism of Chinese herbal medicine in prevention and treatment of cardiovascular diseases. This review discussed 12 kinds of Chinese herbal monomers and 5 kinds of Chinese herbal compounds which have been shown to treat MF by interfering with ncRNAs (Table 1). Through targeting ncRNAs, mainly including miRNA, lncRNA and circRNA, those herbal medicine relieves MF by inhibiting the proliferation/activation/inflammation of CFs, increasing apoptosis/autophagy of CFs, inhibiting apoptosis/inflammation/oxidative stress of cardiomyocytes, increasing viability of cardiomyocytes, repressing EndMT, and promoting myocardium angiogenesis.

At present, however, several challenges persist in the investigation of ncRNAs' role in the anti-MF effect of Chinese herbal medicine: (1) The regulatory effect of Chinese herbal medicine on ncRNAs is primarily validated in animal and cell models, and it is not yet guaranteed whether these effects still exist in complex human bodies. (2) The most reported ncRNA targets of Chinese herbal medicine are miRNAs. Whether lncRNA, circRNA, and other ncRNAs act as key mediators of Chinese herbal medicine's effect has not been explored sufficiently. (3) We noted that some Chinese herbal medicine could target ncRNAs known to be associated with MF. However, there is currently no direct evidence to support the idea that these herbal medicines can relieve MF by modulating these ncRNAs. Further experiments are required to validate these scientific hypotheses.

NcRNAs have been implicated in various diseases and serve as key targets for disease treatment. However, the clinical transformation of RNA-based therapies is hindered by problems related to specificity, delivery and tolerance. Specificity problems indicate undesirable targeting effects caused by uptake of cells other than the target cells, and off-target effects caused by sequence similarity or overdose to a level much higher than endogenous expectations (Sledz and Williams, 2004; Yu et al., 2020). In addition, there is a lack of delivery vectors suitable for delivering ncRNAs to target organs and cell types (Krieg, 2011). What's more, natural RNA molecules are highly susceptible to enzymatic degradation by serum and cellular RNases. Notably, both single-stranded and double-stranded RNAs can trigger the body's viral defense system via pathogen-associated molecular patter (PAMP) receptors (Kumar et al., 2011). Due to these reasons, RNA-based therapies are often lack of efficiency in clinical trials. Therefore, targeting specific ncRNAs with small molecules displays potential as a therapeutic approach for disease treatment. Developing effective tools to screen small molecules against particular ncRNAs is very important and urgent. Recently, a study published in Nature has introduced an innovative technique for screening small molecules that bind to ncRNAs. They devised an unbiased screen based on affinity-selection mass spectrometry to identify reversibly binding ability between lncRNA Xist and 50,000 compounds. They found 20 analogues that has similar structure with the one positive hit and finally obtained one positive compound X1 that can effectively reguate the function of Xist (Aguilar et al., 2022). This research broadens the scope of ncRNA pharmaceutical field, which will enable the development of RNA-targeting drugs by high-throughput and large-scale screening methods. Therefore, by utilizing this screening system with MF-related ncRNAs as binding targets, it holds the promise of identifying a greater number of anti-MF drugs, including Chinese herbal medicine. For example, it has been shown that miR-21 activates the ERK signaling pathway, EndMT, and the TGF-β signaling pathway to promote MF (Cheng et al., 2016; Yuan et al., 2017; Li et al., 2020). Based on the preceding statement, miR-21 has been proven to be a target for many Chinese herbal medicines in combating MF. Tripterine, Notoginsenoside R1, Shenzhu Xinkang Decoction, and the ultrafiltration extract of Radix Angelica Sinensis and Radix Hedysari can all alleviate MF by inhibiting miR-21. Therefore, miR-21 may serve as a binding target for screening anti-MF drugs.

In conclusion, it is clear in this emerging field that ncRNAs appear to be important players in mediating Chinese herbal medicine's effect in various diseases. Indeed, as we have reviewed, ncRNAs interact with fibrosis-related genes and signalling pathways, making them a pivotal bridge in mediating the therapeutic effect of Chinese herbal medicine on MF. Furthermore, ncRNAs represent promising clinical drug targets and establishing an anti-MF drug screening platform based on ncRNAs to screen drugs including Chinese herbal medicine represents a challenging but promising field for future drug development in cardiovascular diseases.

Author contributions

MW: Conceptualization, Data curation, Writing–original draft. MY: Writing–original draft. LT: Writing–original draft, Data curation. XZ: Data curation, Writing–original draft, Methodology. GL: Data curation, Writing–original draft, Visualization. ZZ: Data curation, Writing–original draft. JZ: Writing–review and editing. HG: Writing–review and editing, Methodology, Software, Validation. WQ: Writing–review and editing, Funding acquisition, Supervision, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

AGO	Argonute
ALA	α-linolenic acid
AMPK	AMP-activated protein kinase
ACTG	gamma-actin
ceRNA	competitive endogenous RNA
Cfl2	cofilin-2
CFs	cardiac fibroblasts
circRNA	circular RNA
CTGF	connective tissue growth factor
dsRBP	dsRNA binding protein
ECM	extracellular matrix
EndMT	endothelial to mesenchymal transition
FGFR2	fibroblast growth factor receptor 2
FOXO3	forkhead box O3
FUS	fused in sarcoma
Fbln2	fibullin-2
HOTAIR	HOX transcript antisense RNA
HSRL	human-specific regulatory loci
lncRNA	long non-coding RNA
LOXL3	lysyl oxidase-like protein-3
MALAT1	metastasis associated lung adenocarcinoma transcript 1
Meg3	maternally expressed gene 3
METTL3	methyltransferase like 3
MIAT	myocardial infarction-associated transcript
MF	myocardial fibrosis
miRNA	microRNA
MMP2	matrix metalloproteinase 2
MMP9	matrix metalloproteinase 9
MMP14	matrix metalloproteinase 14
MDM2	murine double minute 2
mTOR	mammalian target of rapamycin
ncRNAs	non-coding RNAs
OGD	oxygen and glucose deprivation
PAMP	pathogen-associated molecular pattern
PHLPP2	PH domain leucine-rich repeat protein phosphatase 2
pre-miRNA	precursor miRNA
PTBP1	polypyrimidine tract-binding protein 1
PRG4	proteoglycan 4
RBP	RNA binding protein
RISC	RNA-inducing silencing complex
SEPP1	selenoprotein P1
Sfrp2	secreted frizzled related protein 2
SNX9	sorting connexin 9
SWI/SNF	switch/sucrose non-fermentable
TAGLN2	transgelin 2
TCF7	transcription factor 7
TIMP	tissue inhibitors of matrix metalloproteinase
TIMP1	tissue inhibitors of matrix metalloproteinase 1
TIMP4	tissue inhibitors of matrix metalloproteinase 4
TMP4	tropomyosin-4
TRPM7	transient receptor potential melastatin 7
VEGF	vascular endothelial growth factor

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EDITED BY
Ismail Laher,
University of British Columbia, Canada

REVIEWED BY
Madeline Nieves-Cintron,
University of California, Davis, United States
Cristina Ribas Fürstenau,
Federal University of ABC, Brazil
Alexey V. Glukhov,
University of Wisconsin-Madison, United States

*CORRESPONDENCE
Shyamala Dakshinamurti,
shyamala.dakshinamurti@umanitoba.ca

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Adenylyl cyclase isoforms 5 and 6 in the cardiovascular system: complex regulation and divergent roles

Saeid Maghsoudi[1,2], Rabia Shuaib[2], Ben Van Bastelaere[2] and Shyamala Dakshinamurti[1,2,3]*

[1]Department of Physiology and Pathophysiology, University of Manitoba, Winnipeg, MB, Canada, [2]Biology of Breathing Group, Children's Hospital Research Institute of Manitoba, Winnipeg, MB, Canada, [3]Section of Neonatology, Department of Pediatrics, Health Sciences Centre, Winnipeg, MB, Canada

Adenylyl cyclases (ACs) are crucial effector enzymes that transduce divergent signals from upstream receptor pathways and are responsible for catalyzing the conversion of ATP to cAMP. The ten AC isoforms are categorized into four main groups; the class III or calcium-inhibited family of ACs comprises AC5 and AC6. These enzymes are very closely related in structure and have a paucity of selective activators or inhibitors, making it difficult to distinguish them experimentally. AC5 and AC6 are highly expressed in the heart and vasculature, as well as the spinal cord and brain; AC6 is also abundant in the lungs, kidney, and liver. However, while AC5 and AC6 have similar expression patterns with some redundant functions, they have distinct physiological roles due to differing regulation and cAMP signaling compartmentation. AC5 is critical in cardiac and vascular function; AC6 is a key effector of vasodilatory pathways in vascular myocytes and is enriched in fetal/neonatal tissues. Expression of both AC5 and AC6 decreases in heart failure; however, AC5 disruption is cardio-protective, while overexpression of AC6 rescues cardiac function in cardiac injury. This is a comprehensive review of the complex regulation of AC5 and AC6 in the cardiovascular system, highlighting overexpression and knockout studies as well as transgenic models illuminating each enzyme and focusing on post-translational modifications that regulate their cellular localization and biological functions. We also describe pharmacological challenges in the design of isoform-selective activators or inhibitors for AC5 and AC6, which may be relevant to developing new therapeutic approaches for several cardiovascular diseases.

KEYWORDS

adenylyl cyclase, G protein-coupled receptors, cyclic 3′,5′-adenosine monophosphate, signal transduction, heart disease, drug discovery

Introduction

Over the course of life, heart health and aging have been correlated with diminished left ventricular (LV) function and weakened cardiac β-adrenergic receptor (βAR) responsiveness. The reasons for this dysfunctionality may include phenotype changes in the LV, reduced βAR density, upregulation of regulatory proteins Gαi and G-protein coupled receptor kinases, or abnormalities in the βAR signaling system (Ferrara et al., 2014). Adenylyl cyclases (ACs), as the central effector molecules for βAR signaling, play a crucial

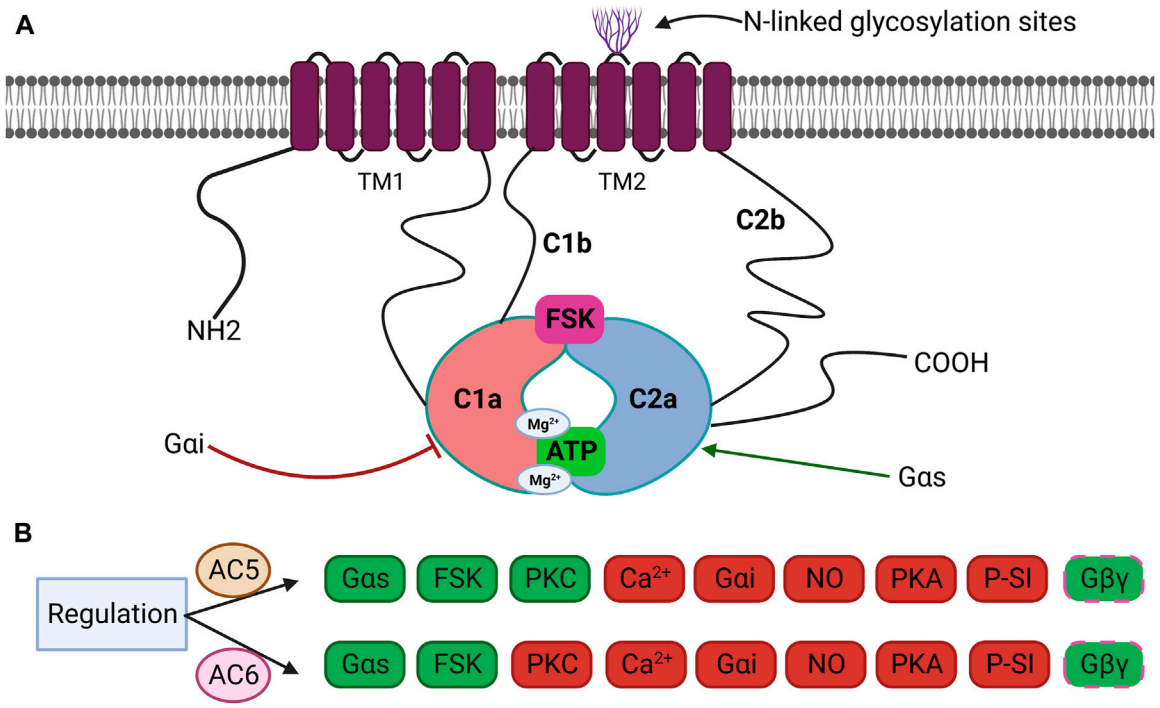

FIGURE 1
(A) Schematic structure of transmembrane adenylyl cyclases (TMACs). TMACs contain an intracellular N-terminus, two repetitions of six TM1 and TM2, and two cytoplasmic domains (C1 and C2) further divided into C1a and C2a. C1a–C2a forms the catalytic domain and FSK-binding site. Inhibitor Gαi binds to C1a, whereas activator Gαs binds to C2a. C1b and C2b are regulatory subdomains, and the N-terminus participates in several protein–protein interactions. Some isoforms are glycosylated on extracellular loops 5 or 6. **(B)** Specific regulation of AC5 and AC6 by various effectors. Both isoforms are fully activated by Gαs and FSK and partially or conditionally activated by Gβγ. AC5 is activated by PKC, whereas AC6 in inhibited by PKC. Both isoforms are inhibited by Ca^{2+}, Gαi, NO, PKA, and P-site inhibitors (P-SI). Green: stimulation, red: inhibition, and dashed line: conditionally.

role in cardiac contractility, relaxation, and LV diastolic function (Tang et al., 2011). The failing heart has poor AC signaling and decreased LV cAMP production, leading to impaired βAR responsiveness to ligands (White et al., 1994; Roth et al., 2002). Persistent activation of the sympatho-adrenergic system in patients with congestive heart failure can also lead to unfavorable cardiac remodeling due to cardiomyocyte loss and fibrotic replacement (Zhang et al., 2013). The distribution of AC isoforms varies within cardiac tissues; ACs 2, 3, 4, 5, 6, and 7 are expressed by cardiac fibroblasts (Ostrom et al., 2003), while AC1 and AC8 are found in sinoatrial node (SAN) cells (Mattick et al., 2007; Younes et al., 2008; Robinson et al., 2021), and AC5 and AC6 are the major AC isoforms expressed in the adult ventricle (Göttle et al., 2009; Efendiev and Dessauer, 2011). AC6 also serves as the principal effector of vasodilator signaling and a regulator of membrane potential in vascular myocytes (Nelson et al., 2011). In this review, we examine the similarities and differences between ACs 5 and 6 in cardiovascular function, highlighting their divergent functional roles and opportunities for pharmacological targeting.

Overview of adenylyl cyclases

Cyclic 3′,5′-adenosine monophosphate (cAMP), a ubiquitous second messenger that mediates a variety of cellular responses, is generated by more than thousand nucleotidyl cyclase proteins classified into six groups according to the amino acid sequence of the catalytic domain (Kamenetsky et al., 2006; Seifert and Beste, 2012). The class III cyclases including eukaryotic adenylyl cyclases (ACs) are indispensable effectors of cAMP (Linder, 2006). Enumerated in order of discovery, mammalian ACs 1–9 (~120–140 KDa) have been identified as transmembrane adenylyl cyclases (TMACs), while AC10 is a soluble enzyme. ACs are canonically regulated by heterotrimeric G-proteins, Gαi and Gαs, upon stimulation of G protein-coupled receptors (GPCRs) (Halls and Cooper, 2017). There are significant variations in the distribution and biochemical characteristics among AC isoforms, as well as distinctive chromosomal loci of individual isoforms (Vatner et al., 2013). The TMACs share a similar framework but different regulation (Sunahara et al., 1996). Despite arising from distinct transcripts, they own a similar overall structure with highly homologous active sites, making it challenging to design selective activators or inhibitors and to differentiate their functions in the organs and tissues (Rana et al., 2017). The mammalian ACs share an intracellular N-terminus, two membrane-spanning domains (TM_1 and TM_2), and conserved catalytic domains, C1 and C2 (each ~40 KDa), together forming the catalytic core. The C1 and C2 cytoplasmic domains are further divided into the highly conserved catalytic regions, C1a and C2a, and the less conserved regulatory regions, C1b and C2b. Two active sites are shaped by their interface: the ATP catalytic site and forskolin (FSK) binding pocket (Figure 1A)

(Schmid et al., 2014; Antoni, 2020). Crystallographic studies have revealed that the hydrophobic pocket generated by C1 and C2 catalytic subunits is the allosteric site for the interaction of FSK with ACs (Pavan et al., 2009; Bhatia et al., 2023). Despite the sequence similarity of the regulatory domains in C1–C2, various signals such as G-proteins, kinases, FSK, and Ca^{2+} uniquely regulate AC isoforms (Brand et al., 2013). Indeed, the C1–C2 domain, at the cytoplasmic surface of the molecule, accommodates binding sites for Gαs, Gαi, FSK, ATP, Mg^{2+}, the regulator of G protein signaling (RGAS2), proteins associated with Myc (PAM), Snapin, Ric8a, A-kinase-anchoring protein (AKAP79), PH domain leucine-rich protein phosphatase 2 (PHLPP2), and phosphorylation and dephosphorylation sites for protein kinase A (PKA) and protein kinase C (PKC) (Sunahara et al., 1997). The crystal structures of the complex made up of the C1 cyclase homology domain (CHD) from AC5 and the C2 CHD from AC2 (VC_1:IIC_2) bound to FSK and GTP-S-activated Gαs served as the fundamental model for the prototype AC catalytic core (Mou et al., 2009). Binding sites for cofactors Mg^{2+} and Mn^{2+} are further located in the active site of AC and must be occupied for catalytic activity, based on structural analyses of activated VC_1:IIC_2 complexes coupled to substrate analogs (Sinha and Sprang, 2006; Mou et al., 2009). Forskolin, a natural diterpene, specifically binds opposite to the ATP-binding site within the catalytic core, using a mixture of hydrophobic and hydrogen interactions to approximate the two cytoplasmic domains, resulting in increased enzyme activity regardless of Gαs docking. No endogenous ligand has yet been identified for the FSK-binding site (Pavan et al., 2009; Dessauer et al., 2017; Bhatia et al., 2023).

While the C1–C2 framing the catalytic core remains highly conserved among individual AC isoforms, the N-terminus is significantly varied across isoforms (Antoni, 2020). The length and composition of the N- and C-terminal domains of ACs are significantly variable outside of the non-conserved domains (Sunahara et al., 1996). The N-terminus of AC1 to AC5 spans 60 to 240 residues in length. Pharmacologically, these are the regions where type-specific regulation of ACs can occur (Halls and Cooper, 2017). The TM domains are not required for catalytic activity since C1a and C2a can be individually shaped into active enzymes by Gαs and FSK (Tang and Gilman, 1995). Removing TM domains and fusing just C1 with C2 make a small but versatile protein which can be used to replicate numerous beneficial effects of AC (Tan et al., 2019). However, the TM regions play important roles in balancing the stoichiometry of their relative catalytic domains (Sunahara et al., 1996), in regulating functional assembly and trafficking of AC (Gu et al., 2002), and may also function as receptors for extracellular signals (Seth et al., 2020).

From a regulatory standpoint, the mammalian AC isoforms are classified into four groups: *Group I*, ACs 1, 3, and 8, stimulated by calcium/calmodulin (Ca^{2+}/CaM); *Group II*, ACs 2, 4, and 7, stimulated by Gβγ; *Group III*, ACs 5 and 6, inhibited by Gαi/Ca^{2+}; and *Group IV*, AC9, which is partially stimulated by FSK (Ostrom et al., 2022). All isoforms are activated by Gαs while differentially regulated by Gαi, Gβγ, and protein kinases (PKA, PKC, Raf-1, and CaM kinases) (Figure 1B) (Antoni, 2020). Most cells and tissues express multiple AC isoforms; however, their varying abundance in specific tissues is noteworthy (Halls and Cooper, 2017; Ostrom et al., 2022).

Role of ACs in cardiac tissue

Key cardiac isoforms AC5 and AC6, known as the Ca^{2+}-inhibited family of ACs, share about 91.5% sequence alignment, except for the AC6 N-terminus (aa 1–86) (Wu et al., 2017). These two isoforms have considerable similarities in their expression patterns and functions, despite their evolution at independent gene loci; the AC5 gene is on chromosome 3 at position 3q13.2–3q21, and AC6 is on chromosome 12 at position 12q12–12q13 (Haber et al., 1994). The canine (type V-a) and the rat (type V-b) forms of AC5 are in fact mRNA splice variants, co-expressed in both species (Iwami et al., 1995). Both AC5 and AC6 are non-competitively inhibited by Gαi at C1a, opposite from the docking site for Gαs at C2a (Taussig et al., 1994). Their tissue distribution and developmental mRNA expression patterns have been studied in several species, including humans (Tobise et al., 1994; Espinasse et al., 1995; Wang and Brown, 2004). AC6 is more expressed in the neonatal heart, while AC5 appears to be predominantly an adult isoform (Espinasse et al., 1995). The maximal amount of AC6 mRNA is expressed during fetal development, while steadily diminishing with age, and reaching its lowest amount in fully mature adults. On the contrary, a minimal amount of AC5 mRNA is expressed during fetal development, but progressively increasing with age, and reaching its highest point in the fully developed adult (Tobise et al., 1994; Okumura et al., 2009). In the mouse heart, the abundance of AC5 protein reaches its highest point before 1 week and then decreases to levels similar to those of mice studied at 3, 6, and 24 months of age. In the rat heart, however, the AC5 protein level peaks before 2 weeks of age and subsequently decreases by 3 months. In pigs, cardiac AC5 protein abundance is high in 1-day-old hearts and then diminishes with age, reaching its lowest content in the heart at 5–6 months (Hu et al., 2009). Feedback regulation of AC expression also varies as a function of age; PDE3 inhibition (PDE3i) has been found to alter AC expression levels in adult and pediatric subjects with dilated cardiomyopathy (DCM) and in pediatric subjects with a congenital single right ventricle. These groups demonstrated distinctive AC isoform mRNA expression patterns. Compared to the non-failing adult LV myocardium, the adult DCM myocardium exhibits upregulation of AC6; chronic PDE3i treatment enhances mRNA expression of AC5 and AC6, while all other isoforms are expressed at levels comparable to those of DCM patients not receiving PDE3i treatment. In contrast, the non-failing pediatric right ventricle expresses less AC5 than does the pediatric single ventricle; and in pediatric DCM, AC5 and AC6 remained unchanged by PDE3i (Nakano et al., 2017). Due to their structural homology and the paucity of type-specific antibodies, definitive levels of protein expression in the heart have been difficult to establish (Hu et al., 2009). Even following the deletion of AC5, 60% of immunodetectable AC5/6 was observed in cardiac myocytes, suggesting some degree of epitope overlap (Okumura et al., 2003b).

To distinguish their specific roles in cardiac function, AC5 and AC6 have been the subject of several transgenic overexpression and deletion studies. Both isoforms are highly expressed in the heart and

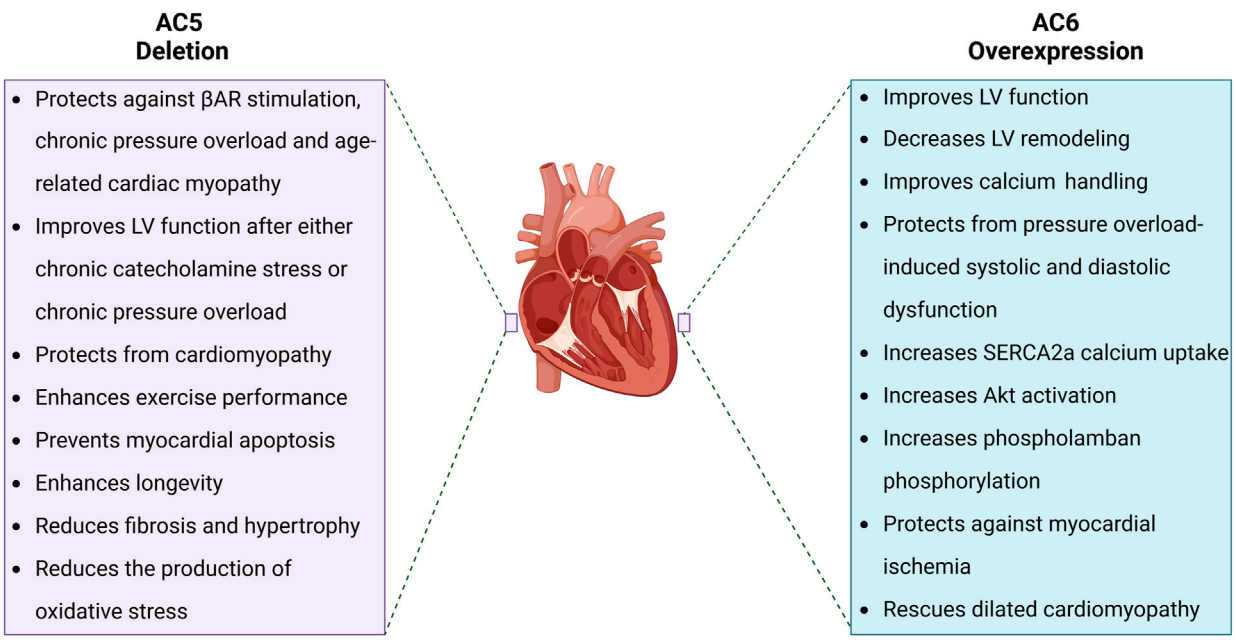

FIGURE 2
Pathophysiological effects of AC5 deletion and AC6 overexpression. AC5 and AC6 behave distinctly when increased or disrupted. While AC5 deletion has come with several beneficial effects for the heart such as prolonged longevity and enhanced LV function, AC6 overexpression improves cardiac cAMP generation and Ca^{2+} handling, resulting in improved LV function.

are important negative feedback responses of cardiac rhythmicity (Cosson et al., 2019). While both isoforms regulate heart rate (HR) and contractility, AC6 appears more important at baseline cardiac function (Vatner et al., 2013). AC5 and AC6 may function distinctly in the pathogenesis of cardiac stress responses. The protein abundance of AC5 and AC6 responds oppositely to pressure overload LV hypertrophy (Okumura et al., 2003b; Sugano et al., 2011), with upregulation of AC5 and downregulation of AC6, implying that it is AC5 that plays a role in chronic pressure overload cardiomyopathy (Vatner et al., 2013). It is probable that these two isoforms also play different physiological roles in regulating cardiac function during dilated cardiomyopathy. Both AC5 and AC6 mRNAs appear decreased in dogs with pacing-induced Congestive Heart Failure (CHF) (Ishikawa et al., 1994); while in a swine model of tachypacing and severe heart failure, AC6 but not AC5 is downregulated (Ping et al., 1997). While some studies suggest AC5 overexpression improves cardiac function in transgenic mice during exercise (Esposito et al., 2008), deletion of AC5 protects the heart in most models of cardiac stress including heart failure; in contrast, AC6 overexpression has been correlated with cardioprotective effects (Wu et al., 2017; Ostrom et al., 2022). The overexpression of AC6 leads to increased LV function, improved cAMP and Ca^{2+} handling, and may protect the heart from pressure overload-induced systolic and diastolic dysfunction (Phan et al., 2007; Guellich et al., 2010). AC5 deletion, on the other hand, results in protecting the heart from cardiomyopathy, chronic catecholamine stress, and chronic pressure overload (Okumura et al., 2003b; Okumura et al., 2007). Isoform-specific effects are not restricted to myocardial AC5 and AC6. Cardiac-specific overexpression of AC8 in 3-month-old transgenic mice alters several pathways, resulting in elevated AC activity and cAMP-induced cardiac workload until up to 1 year of age, without excess mortality or onset of heart failure (Tarasov et al., 2022; Qu et al., 2024). However, other studies have shown cardiac overexpression of AC8 to cause early and accelerated cardiac remodeling, resulting in development of heart failure and a shortened life span, suggesting that alterations in cAMP/PKA signaling can hasten cardiac aging, in part via the glycogen-synthase-kinase 3α/β (GSK3α/β) phosphorylation pathway (Mougenot et al., 2019).

Although there remains some controversy regarding the specific regulation and roles of AC5 and AC6 isoforms in controlling cardiac function, their roles in the production of required cAMP to initiate cardiac chronotropic and inotropic responses are indispensable. It is thus important to differentiate the regulation and biological functions of AC5 *versus* AC6, leading the way to development of targeted treatments for different cardiac pathologies. The respective therapeutic outcomes of AC5 deletion and AC6 overexpression in the cardiovascular system are included in Figure 2.

Regulation of AC5 and AC6 by calcium

Ca^{2+} serves as a pivotal regulator of AC5 and AC6, playing a crucial role in the control of cellular homeostasis and heart function regulation. The inhibition of ACs 5 and 6 by Ca^{2+}, which is non-competitive with respect to ATP (Guillou et al., 1999), contributes to pacemaking in cardiac tissue and also sustains endothelial cell permeability. Inhibition of these cardiac AC isoforms by Ca^{2+}, which their own cAMP generation permits to enter the cardiomyocyte, serves as a form of feedback control regulating pacemaker rhythmicity as well as the force of contraction (Mou

et al., 2009). A wide range of *in vitro* inhibitory sensitivities are reported. Systolic [Ca^{2+}] 1 μM is required to inhibit the activity of AC5/6, while at higher intracellular [Ca^{2+}] (10–25 μM), the activity of all AC isoforms is inhibited (Guillou et al., 1999; Halls and Cooper, 2017). However, AC5 and AC6 inhibition by Ca^{2+} is biphasic, involving low- and high-affinity binding sites, so Ca^{2+} can inhibit AC5 and AC6 in sub- to supramicromolar concentration ranges (Steer and Levitzki, 1975; HANSKI et al., 1977; OLDHAM et al., 1984; Fagan et al., 1998; Guillou et al., 1999). The sub-micromolar [Ca^{2+}] (0.2–0.6 μM) inhibits AC5 and AC6 in membrane preparations from different tissues, cultured cell lines, and in recombinant systems (Beazely and Watts, 2006). Mechanistically, Ca^{2+} antagonizes the activation of AC5 and AC6 by Mg^{2+} (Mou et al., 2009). Site-directed mutations of AC5 in the presence of other divalent cations indicate that the Mg^{2+}-binding loci at the AC5 catalytic site are essential for its Ca^{2+}-mediated inhibition (Hu et al., 2002; Mou et al., 2009). In addition to being a hub for GPCR AC interactions, lipid rafts are locations for capacitive Ca^{2+} entry channels; *Group III* AC isoforms that are localized in lipid rafts are also regulated by Ca^{2+} (Ostrom and Insel, 2004). Disruption of lipid rafts results in loss of capacitive Ca^{2+} entry and dysregulation of Ca^{2+}-regulated AC isoforms (Dessauer et al., 2017).

Subcellular localization of AC5 and AC6

GPCR signaling pathways are type-specific for either AC5 or AC6, as determined by the colocalization of receptors and AC isoforms in lipid raft or non-raft plasma membranes (Ostrom et al., 2000). ACs 4–7 are expressed in the heart and vascular system, but among them, AC5 and AC6 are dominant subtypes in adult mammalian heart (Dessauer et al., 2017). The compartmentation of AC5 and AC6 in caveolae or membrane lipid rafts provides a key biochemical process for temporal and spatial segregation of signal transduction as well as cross-talk between signaling cascades, resulting in the compartmentalization of cAMP signaling (Thangavel et al., 2009). While native AC5/6 are found in caveolar fractions, overexpressed AC6 in rat aortic smooth muscle cells is found in non-caveolin-rich fractions, where it is functional and increases cAMP (Ostrom et al., 2002). In these cells, low levels of AC6 overexpression do not much change cAMP levels at baseline nor responses to adenosine A2b receptor challenge (Ostrom et al., 2002; Thangavel et al., 2009). In contrast, in cardiomyocytes, overexpressed AC6 localizes similarly to endogenous AC6, which is targeted to caveolae, demonstrating that GPCR and AC5/6 compartmentation to caveolin-rich membranes is cell-type dependent (Ostrom et al., 2000; Ostrom et al., 2001; Ostrom et al., 2002). Overexpression of AC6 improves βAR responses without affecting signaling of other Gαs-coupled receptors in a variety of cells, including airway smooth muscle, lung fibroblasts, and neonatal myocytes (Sunahara et al., 1996; Liu et al., 2008; Sadana and Dessauer, 2009; Dessauer et al., 2017). In vascular smooth muscle cells (VSMCs), AC3, AC5, and AC6 are the most abundant isoforms in βAR-mediated signaling, but during vasodilation, AC6 is the principal isoform involved in βAR-mediated cAMP/PKA signaling and activation of the ATP-sensitive potassium current, thus playing an important role in setting the membrane potential. Its counterpart AC5 does not have similar activity, (Nelson et al., 2011). In ventricular myocytes, isoforms AC5 and AC6 have separate subcellular compartmentalization. While AC5 is mostly found deep in transverse tubules interacting with caveolin-3 (CAV3) and phosphodiesterases (PDEs), AC6 is located in the plasma membrane outside the t-tubular area (Figure 3) (Timofeyev et al., 2013). βAR receptors are also differentially distributed; β1- and β2AR are found within t-tubules, but β1AR (comprising >70% of cardiac βAR) is found on the sarcolemma outside t-tubules (Brodde, 1991). β1AR triggers cAMP generation, which increases PKA-mediated phosphorylation of L-type Ca^{2+} channels as well as other regulatory proteins, greatly increasing cardiac contractility; β2AR couples with both Gαs and Gαi, resulting in a smaller increment of contractility (Zheng et al., 2005). Inactivation of Gαi by pertussis toxin enhances β1AR-mediated inotropy despite β1AR not coupling to Gαi, indicating that Gαi also inhibits the AC5/6 receptor independently (Cosson et al., 2019). Due to subcellular localization, AC5 has been connected to β2AR signaling, while AC6 is responsible for extra-tubular β1AR signaling as well as β1AR-mediated augmentation of the L-type Ca^{2+} channel current ($I_{Ca,L}$) in ventricular myocytes (Timofeyev et al., 2013) (Figure 3). Both βAR subtypes have obvious inotropic effects; dobutamine, a non-selective βAR agonist, increases cardiac contractility in both AC5KO and AC6KO mice (Tang et al., 2006; Tang et al., 2008). While β2AR overexpression enhances ventricular function and activates cell survival pathways (Milano et al., 1994), β1AR overexpression appears catastrophic, causing both cardiac hypertrophy and dilated cardiomyopathy (Engelhardt et al., 1999). The opposite effects have been reported following manipulation of their more proximal AC subtypes; AC5 disruption in t-tubules is cardioprotective (Okumura et al., 2003b), while overexpression of AC6 rescues heart function in cardiac injury (Gao et al., 2016). However, functional coupling may not only reflect localization. In AC6KO, a shift from dominant AC6 coupling to AC5 coupling occurs in the β1AR signaling cascade. This change in the AC assignment in AC6KO results in rearranged signaling compartmentalization as well as an alteration in the PDE isoform control of the cAMP pool (Cosson et al., 2019; Ostrom et al., 2022). These findings suggest some functional redundancy of AC5 or AC6 for β1AR-mediated inotropic responses. Additionally, in CHF models largely ascribed to excess β1AR activity, β2AR redistributes to the cell surface, and this loss of cAMP compartmentation correlates with heart failure (Nikolaev et al., 2010). Studies of detubulated cardiomyocytes reveal that stimulation of both β1AR and β2AR is more functionally effective at the sarcolemmal surface, rather than within t-tubules (Cros and Brette, 2013). Pharmacological inhibition studies confirm that β1AR preferentially couples with AC5 at t-tubules, while β2AR couples with AC6 when present at the cell surface, resulting in the observed AC subtype-specific effects on cardiac function and survival signaling (Tsunematsu et al., 2015).

Compartmentalization of cAMP signaling in the heart

While most tissues express a plethora of AC isoforms (Figure 4), the specific roles of each isoform may overlap within that cell type (Sadana and Dessauer, 2009). Functional redundancy or compensatory roles have been proposed within individual AC groups, as they are regulated or expressed in a comparable manner (Defer et al., 2000). Changes in AC activity may depend

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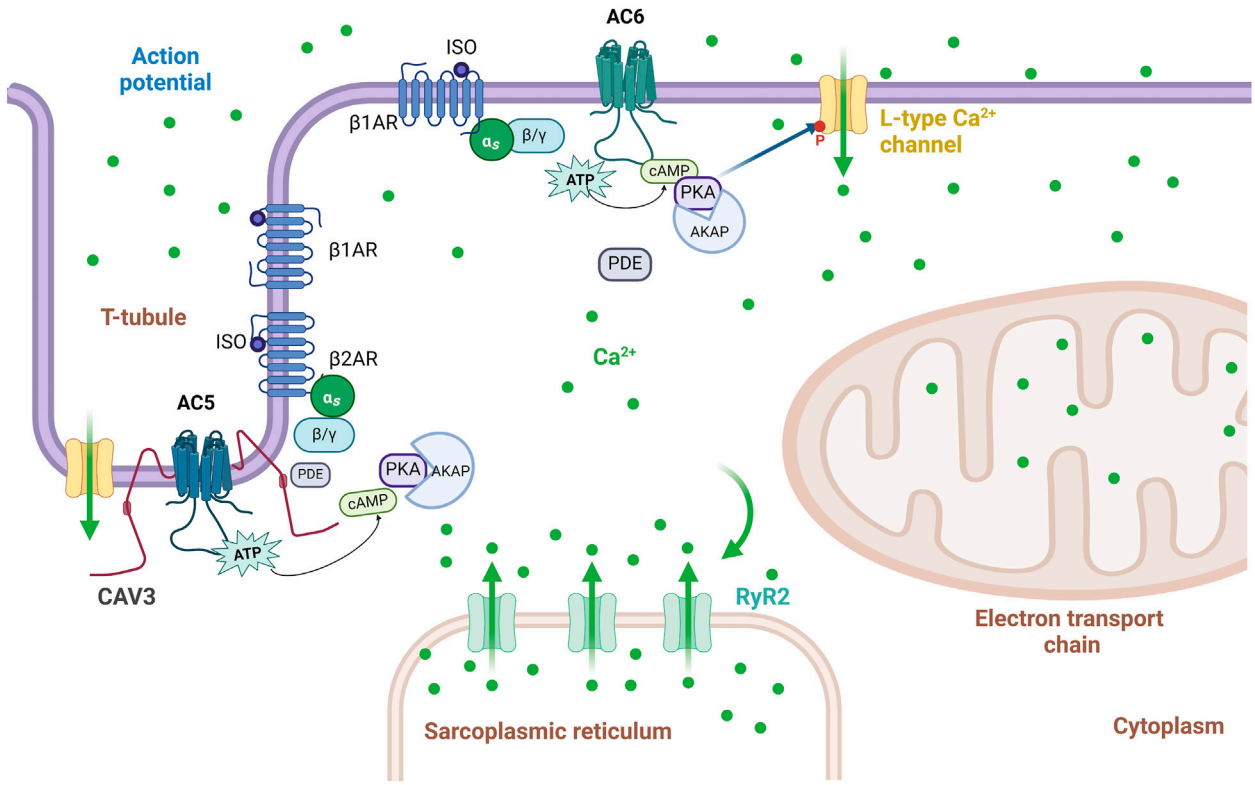

FIGURE 3
Schematic representation of the localization of AC5 and AC6 signaling within the t-tubule and sarcolemmal membrane. In ventricular myocytes, AC6 is localized to the plasma membrane outside of the t-tubular region, and it interacts only with β1AR signaling-mediated augmentation of the L-type Ca^{2+} current (ICa,L), while AC5 is localized to the membrane in t-tubular regions, and its function on ICa,L is restrained by cAMP degradation by phosphodiesterases. Adapted from (Timofeyev et al., 2013); image designed using BioRender.

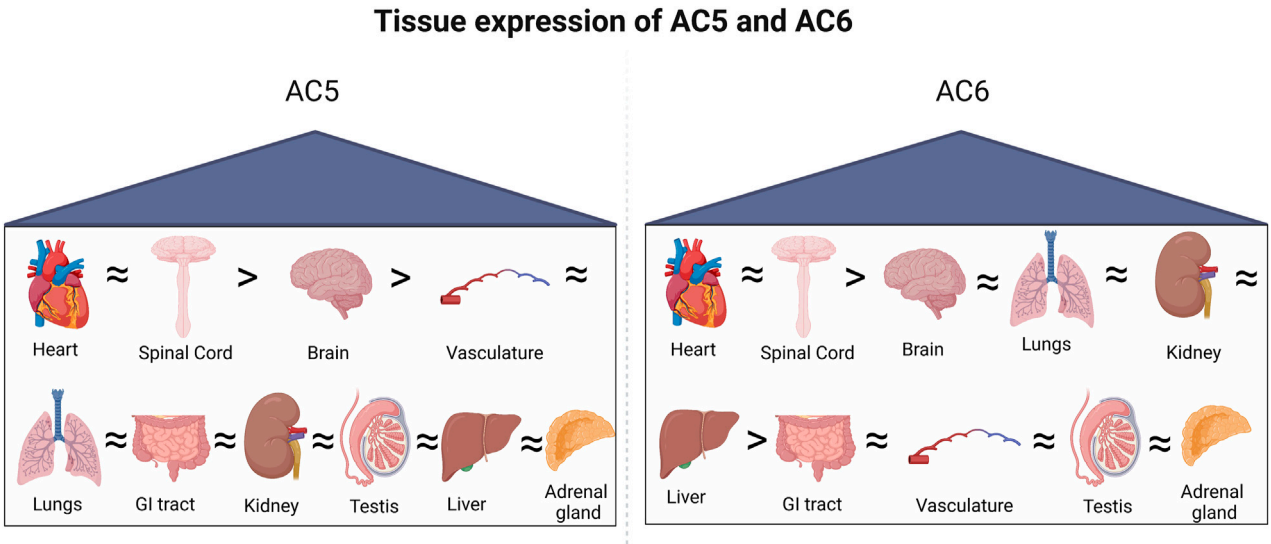

FIGURE 4
Tissue-specific expression of AC5 and AC6. Both isoforms are highly expressed in the heart and spinal cord, followed by the brain.

on the profile of isoforms present in a certain cell or tissue (Tao et al., 1998). Differing phenotypes of AC5 and AC6 activity suggest the maintenance of distinct pools of cAMP within cardiac myocytes, generated by each enzyme. Regulation and localization of AC5 and AC6 isoforms tailor the generation of cAMP by concurrent signals, assisting in dynamic control of the cAMP signal. The

compartmentation of cAMP signaling in the heart arises, in part, from specific signaling complexes which generate distinct cell responses, with AC5 and AC6 being central effectors to the formation and maintenance of these compartments.

The organization of cAMP microdomains in isolated cardiac cells has been researched extensively, using various effector proteins to sort distinct cAMP pools (Patel and Gold, 2015). Several downstream effectors can regulate cAMP production or hydrolysis, including PDEs, PKA, cyclic nucleotide-gated (CNG) channels, hyperpolarization-activated cyclic nucleotide-gated exchange proteins, exchange protein directly activated by cAMP (Epac), and the Popeye domain-containing (POPDC) protein family. At the level of cAMP signal longevity, PDE isoforms, importantly PDE3/4, play pivotal roles in the discrete regulation of localized cAMP levels in neonatal cardiac myocytes (Willoughby and Cooper, 2007). PDE4 isoforms regulate cAMP signaling resulting from β1AR and β2AR stimulation in cardiac myocytes; PDE4B controls β1AR but not β2AR responses in cardiac myocytes, suggesting a localized function to control β1AR-dependent excitation–contraction coupling (Mika et al., 2014). PDE3 acts globally on cAMP in normal and heart failure models, modulating cAMP-mediated regulation of Ca^{2+} re-uptake in the sarcoplasmic reticulum (SR) (Calamera et al., 2022). Spatial and temporal organization of cAMP-dependent pathways is further provided by cAMP-related protein scaffolding, biasing the cAMP signal toward different end functions.

AKAP organizes AC5 and AC6 cAMP in the heart

A-kinase-anchoring proteins (AKAPs) are scaffold proteins which coordinate signaling components into multiprotein complexes, giving rise to regulation of the spatial and temporal organization and fine-tuning of cAMP signaling (Kritzer et al., 2012), ensuring correct targeting of cAMP-dependent PKA and other signaling enzymes to precise subcellular compartments (Diviani et al., 2016). AKAPs function through binding to the regulatory subunits of PKA and crucial PKA phosphorylation sites to initiate rapid and targeted coupling of the kinase to downstream effectors (Ostrom et al., 2022). AC5/6 in the heart utilizes the interactions with AKAPs to cohort with upstream and/or downstream effectors. Over 50 AKAPs are known to target PKA to various cellular locations; important cardiovascular AKAPs include AKAP15/18, AKAP79/150, Yotiao, mAKAP, AKAP-Lbc, and Gravin (Baldwin and Dessauer, 2018). The interaction of these AKAPs with AC5 or AC6 determines the localization and activity of their cAMP signaling complexes (Efendiev and Dessauer, 2011). As scaffolding effectors, AKAPs not only anchor the localization of ACs but also preserve local pools of cAMP by assembling macromolecular complexes (Baldwin and Dessauer, 2018). In cardiomyocytes, the arrangement of MAP kinase, Ca^{2+}, and cAMP-dependent pathways is coordinated through the muscle A-kinase anchoring protein β, playing a key role in cellular hypertrophy (Kapiloff et al., 2009). AC5 binds to a specific N-terminal site on mAKAP-(245–340), resulting in impairment of AC5 activity; interruption of mAKAPβ–AC5 complexes could be targeted therapeutically to reduce cAMP generation and hypertrophy in cardiac myocytes (Bauman et al., 2006; Kapiloff et al., 2009). Both AC5 and AC6 interact with AKAP79/150 along with PKA, generating a negative feedback loop where cAMP production is impaired by PKA phosphorylation of AC5/6 (Baldwin and Dessauer, 2018).

AC5 and AC6 cAMP regulation by Epac in the heart

Another downstream cAMP effector is Epac, a family of cAMP-regulated guanine nucleotide exchange factors functioning independently of PKA as mediators of cAMP signaling. While considered less important in cardiomyocytes than PKA, Epac has high affinity to bind to cAMP, resulting in activation of small GTPases Rap1 and Rap2 (Tan et al., 2022). Epac1 and Epac2 play roles in cardiovascular Ca^{2+} signaling and vascular endothelial barrier formation (Lezoualc'h et al., 2016). AC5 transgenic mice with knockout of the Epac1 gene had decreased cardiac dysfunction and were less susceptible to pacing-induced atrial fibrillation after chronic isoproterenol infusion compared to controls while evincing less cardiac apoptosis and fibrosis than the AC5 transgenics, suggesting that Epac1 mediates the deleterious effects of AC5 overexpression on cardiac function and rhythmogenesis (Cai et al., 2016). On the other hand, mice lacking Epac1 are protected against pressure overload or chronic catecholamine stress-induced cardiac dysfunction (Fujita et al., 2017), reinforcing the notion that rate- and pressure-induced cardiac failure may arise from separate cAMP pools.

Advances in cAMP biosensors in live cells

Various constructs have been developed to measure real-time cAMP levels in living cells or tissues. Previous biochemical methods or radioligand tools were not able to precisely measure the spatiotemporal resolution of intercellular cAMP fluctuations; however, several fluorescent or luminescent biosensors have been introduced that can track real-time cAMP levels in living cells. These cAMP sensors have the advantage of facilitating the study of complex, compartment-specific cAMP-dependent responses (Warrier et al., 2005).

Classical methods of determining AC activity have involved detection of radiolabeled substrate ATP conversion to AMP and detection of enzyme reaction products by chromatography (Streeto and Reddy, 1967; Krishna et al., 1968); these methods can be applied to cell or tissue lysates. Loss of ATP-bound lanthanide fluorescence has been used as an indirect but specific indicator of AC ATPase activity (Spangler et al., 2008b). The terbium–norfloxacin AC activity assay measures the substrate turnover as an assessment of AC catalytic function; varying the exogenous ATP substrate concentration permits the calculation of Michaelis–Menten kinetics (Spangler et al., 2008a). In contrast, intact cell cAMP assays are cumulative measures of product formation in the absence of exogenous substrates, reflecting real-time steady state inclusive of cAMP generation, degradation, or export. Genetically encoded cAMP sensors can also be targeted to subcellular location.

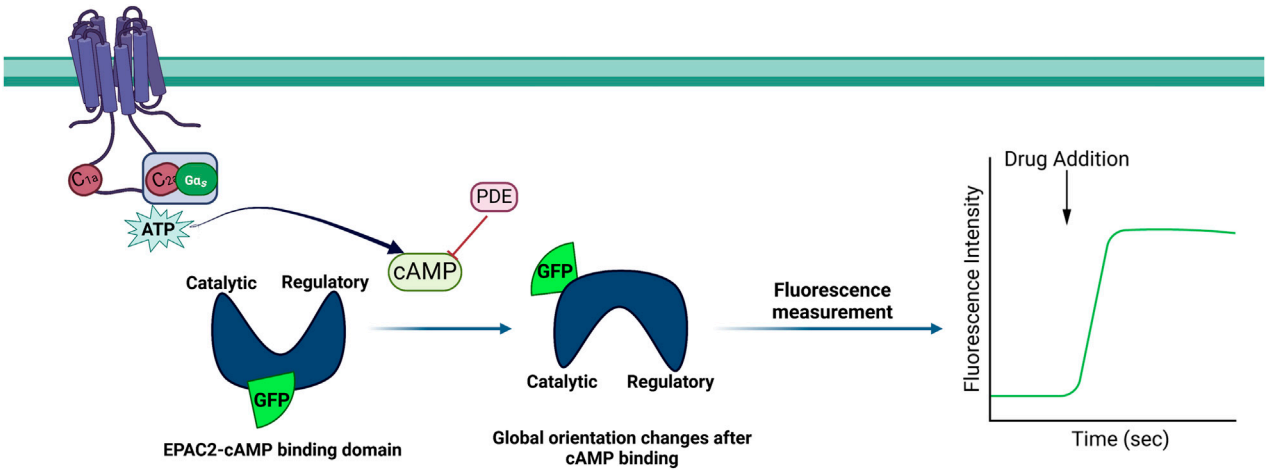

FIGURE 5
Schematic illustration of the cAMP difference detector *in situ* (cADDis) assay. The enzymatic and catalytic domains of the guanine nucleotide exchange factor Epac2 were joined to form circularly permuted green fluorescent protein (cpGFP). The binding and unbinding of the cAMP molecule cause a conformational change in Epac2.

Fluorescence or Förster resonance energy transfer (FRET) uses an excited donor fluorophore to transfer energy to a proximal acceptor fluorophore, which then emits a photon of a lower energy, resulting in detectable red-shift of the emitted light. FRET biosensors used to detect cAMP include PKA-, Epac-, and CNGC-based cAMP sensors. The original FRET PKA-based cAMP biosensor uses fluorescein (donor) and rhodamine (acceptor) to measure the decrease in the FRET signal due to elevation in intracellular cAMP, leading to dissociation of the regulatory subunit of PKA from its catalytic segment (Adams et al., 1991). This sensor was the first to measure microdomain cAMP levels, demonstrating a striated pattern in neonatal rat ventricular cardiomyocytes because of the interaction of PKA with AKAPs near Z-lines. These sensors have also been used to interrogate the roles of PDE3 and PDE4 in cAMP compartmentation in neonatal rat ventricular myocytes (Mongillo et al., 2004). However, the PKA-based biosensor has some limitations: a low signal-to-noise ratio, narrow dynamic range, slow response time, and need for transfection of two distinct constructs (Ponsioen et al., 2004). Epac-based cAMP biosensors address some of these issues. In these, Epac activation is determined in live cells by sandwiching Epac between cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP), taking advantage of a conformational shift generated by cAMP binding. When using cAMP-lowering agonists, FRET fully recovers, but quickly diminishes in response to cAMP-raising agents. As a result, CFP–Epac–YFP is considered a very sensitive cAMP indicator (Ponsioen et al., 2004). Epac-based cAMP biosensors have been used for monitoring Gi/o-mediated cAMP reduction after stimulation of adrenergic α2A or μ opioid receptors in a time-resolved manner without pre-stimulation with FSK or IBMX to increase endogenous cAMP levels (Storch et al., 2017), indicating detection of low levels of cAMP. The use of FRET-based sensors to visualize cAMP signaling microdomains by fluorescence microscopy has been well-reviewed (Calebiro and Maiellaro, 2014).

A newer, versatile approach for measuring cAMP after Gαs- or Gαi-mediated signaling is the cAMP difference detector *in situ* (cADDis) assay (schematic representation, Figure 5). This sensor uses a single fluorescent protein, the Epac cAMP-binding domain fused to circularly permuted mNeonGreen and red mMaple, resulting in a readily detectable bright green fluorescence signal; the Upward cADDis sensor manifests a rise in green fluorescence with increased cAMP, while Downward cADDis decreases fluorescence in response to increased cAMP (Tewson et al., 2018). The cADDis sensor is capable of differentiating basal cAMP from that stimulated by receptor agonism as well as cAMP changes due to Gαs or Gαi activation, when expressed in HEK293T cells (Tewson et al., 2018). Due to its robust expression in multiple cell types and ability to measure real-time cAMP kinetics in live cells over long periods of time with little loss of sensor signals, cADDis has been used in many recent studies of AC cAMP production (Baldwin et al., 2019; Bhatia et al., 2023; Cattani-Cavalieri et al., 2023; Ripoll and Von Zastrow, 2023). Other transfectable single-fluorescent protein cAMP sensors in use include fluorescent cAMP indicator (Flamindo) (Odaka et al., 2014), red fluorescent indicator for cAMP (R-FlincA) (Ohta et al., 2018), and cAMPr (Hackley et al., 2018).

BRET biosensors (bioluminescence resonance energy transfer) use a donor luciferase to oxidize luciferin-generating bioluminescence that excites the acceptor, emitting light at an extended wavelength (Wu and Jiang, 2022). BRET enables detection of fusion protein interactions directly, without the need of an external light source to stimulate a fluorescent energy donor (Prinz et al., 2006). This technology further addresses the problems of photobleaching, autofluorescence, and signal-to-noise ratio in FRET methods; the drawbacks include difficulty of substrate delivery and stoichiometry and potential for cytotoxicity (Wu and Jiang, 2022). BRET-based Epac1-, PKA-, and nano lantern-based cAMP sensors have been developed to investigate cAMP generation (Masri et al., 2008; Hunter et al., 2017; Valkovic et al., 2018).

All plasmid-based cAMP sensors have advantages and disadvantages, with respect to ease of introduction without

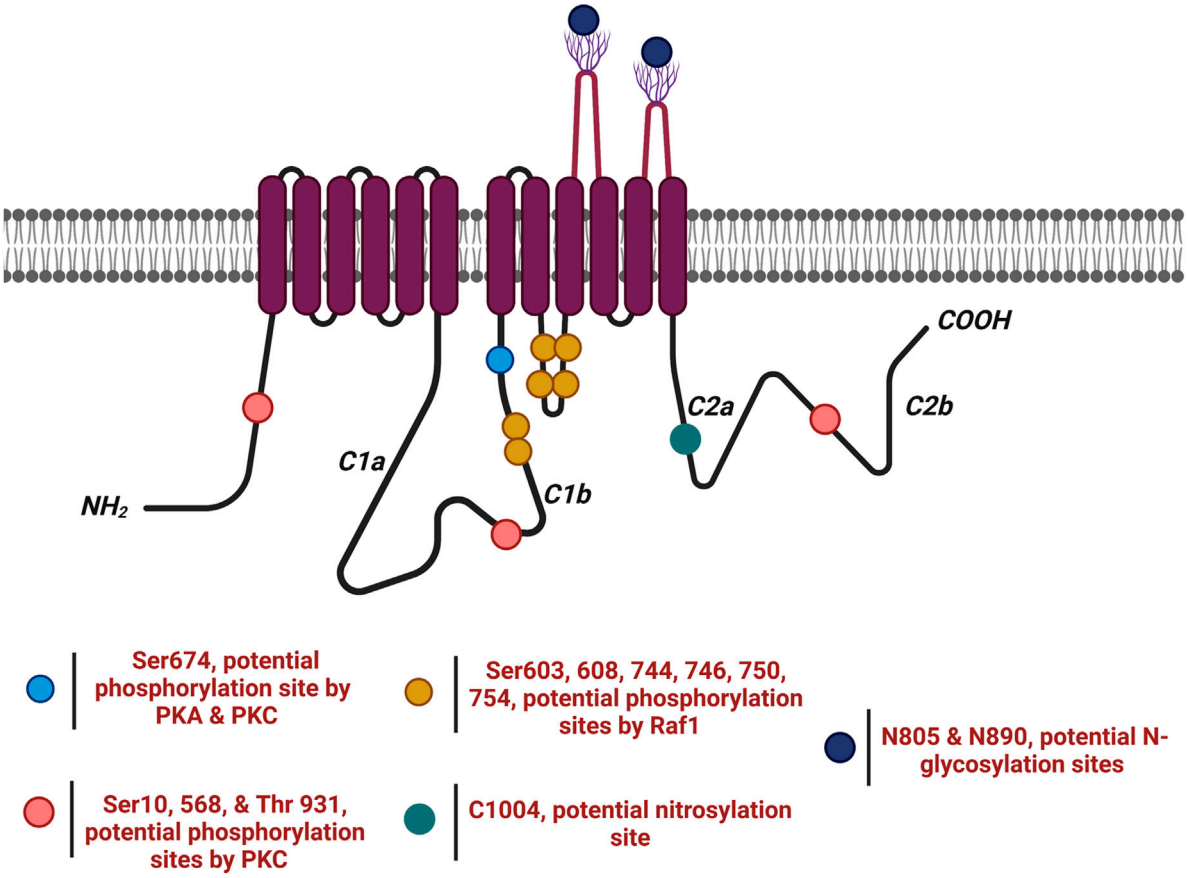

FIGURE 6
AC6 undergoes several post-translational modifications (). AC6 is phosphorylated by PKA and PKC at Ser674 (); phosphorylated by PKC at Ser10 and 568 and Thr931 (); S-nitrosylated by NO at C1004 (); Raf1 on Ser603, 608, 744, 746, 750, and 754 (); and glycosylated at N805 and N890 on extracellular loops 5 and 6.

toxicity, targetability of expression in specific subcellular locations, temporal and spatial resolution of cAMP detection, cAMP detection ranges, and buffering from metabolic processes or post-translational modifications in living cells or tissues. Rapid cAMP binding, instant transmission of signals, and rapid reversibility of the binding event would denote an ideal cAMP sensor (Paramonov et al., 2015). Further optimization is needed for detection of low unstimulated cAMP levels in sensitive primary cells or tissues. Available cAMP biosensors have recently been reviewed in detail (Kim et al., 2021).

Regulatory post-translational modifications of AC5 and AC6

AC5/6 phosphorylation by PKA and PKC

PKA and PKC modify AC5 and AC6 activity by phosphorylating serine (Ser) or threonine (Thr) residues. In the heart, PKA regulates metabolism, gene transcription, ion fluxes, and contraction (Colombe and Pidoux, 2021) and also acts as a feedback inhibitor for both AC5 and AC6 through inhibitory phosphorylation near the end of the C1b domain, resulting in the desensitization of AC activity (Sadana and Dessauer, 2009). Stimulation of AC6 was lost after treatment of PKA even in the presence of high concentrations of active Gαs (Chen et al., 1997). Mutational analysis indicates Ser674 is the target of phosphorylation and inhibition of AC6 by PKA in intact cells (Figure 6) (Beazely and Watts, 2006; Chen et al., 1997). AC5 possesses 14 putative PKA phosphorylation sites, including a Ser 788 that corresponds to Ser674 in AC6; however, a specific PKA phosphorylation site in AC5 has not been confirmed (Iwami et al., 1995; Chen et al., 1997).

While both AC5 and AC6 isoforms are inhibited through phosphorylation by PKA, PKC inhibits only AC6 (Kamide et al., 2015; Liu et al., 2022). Previous studies have shown that AC2, AC3, and AC5 can be stimulated by PKC, while AC6 activity is inhibited (Lai et al., 1997; Defer et al., 2000). Phosphorylation of AC5 by PKCα or PKCζ enhances basal activity as well as FSK- or Gαs-stimulated cAMP accumulation; PKC-ζ phosphorylation of AC5 results in a 20-fold increase in AC activity (Kawabe et al., 1996). In the heart, generation of phosphatidyl-inositol-3,4,5 triphosphate via hormonal or growth factor activation of phosphatidyl-inositol 3-kinase can activate PKC-ζ, thus directly activating AC5 production of cAMP (Hanoune and Defer, 2001). In contrast, PKC activators have either no effect or inhibitory effect

on AC6 activity (Chen and Iyengar, 1993; Jacobowitz et al., 1993; Lai et al., 1997; Lai et al., 1999). Mutational analysis of the N-terminus of AC6 as a regulatory domain showed that elimination of residues from 1 to 86 or a single mutation of Ser10 prevents phosphorylation and inhibition of AC6 by PKC (Figure 6) (Lai et al., 1999). Subsequent studies also identified AC6 C1 Ser 568 and 674 as well as AC6 C2 Thr931 as inhibitory targets of PKC, suggesting that phosphorylation of this complex of Ser and Thr might trigger a conformational change in the catalytic core, changing AC6 catalytic activity (Lin et al., 2002).

AC5/6 regulation by glycosylation

AC6 can be glycosylated on two asparagine residues, namely, N805 and N890, on extracellular loops of TM2 (Figure 6) (Wu et al., 2001). The glycosylation of AC6 results in alteration of not only its catalytic activity but also its regulation by Gαi or by PKC. Inhibition of glycosylation by tunicamycin impairs FSK-stimulated AC6 activity, and mutation of glycosylation sites resulted in significantly lower FSK-, Mn^{2+}-, and Mg^{2+}-stimulated enzyme activities than did wild-type AC6, suggesting that glycosylation may be necessary for maintenance of AC6 activity (Wu et al., 2001). AC5 glycosylation has not been verified.

AC5/6 regulation by nitrosylation

Cardiac function, airway, and vascular tone, as well as regulation of immunological defense and neuronal plasticity, are regulated through nitric oxide or reactive nitrogen species (Bhatia et al., 2021). NO-mediated S-nitrosylation of AC5/AC6 plays a crucial regulatory role in physiological processes of cardiovascular function. Previous studies showed that NO, independent of its action on the guanylyl cyclase (GC) pathway, inhibits cAMP in *Dictyostelium discoideum*, indicating that NO can have a direct regulatory effect on ACs via modification of an AC regulatory domain (McVey et al., 1999). Agonist-stimulated cAMP accumulation is inhibited when N18TG2 neuroblastoma cells (Tao et al., 1998) or cardiac myocytes (Joe et al., 1998; Vila-Petroff et al., 1999) are treated with NO or NO donors (Tao et al., 1998). This inhibition is not dependent on the effect of Gαi (McVey et al., 1999) nor on PDE activity (Watson et al., 2001). NO or NO donors were shown to selectively decrease FSK-stimulated AC5/6 activity but not AC1 or AC2 (Hill et al., 2000), while calmodulin stimulation of AC1 is inhibited by NO (Duhe et al., 1994). NO suppression of hormonal or FSK-stimulated AC activity in neuroblastoma plasma membranes does not require CaM (McVey et al., 1999) and directly inhibits FSK-stimulated AC5 and AC6 activity (Hill et al., 2000).

The presence of caveolae, coincidentally the location of endogenous NO generation by endothelial nitric oxide synthase (eNOS), is required for NO inhibition of AC6 activity. Inhibition of AC5/6 by NO relies upon their localization in lipid rafts with caveolin signaling complexes (Ostrom et al., 2004). While lipid raft depletion with β-cyclodextrin prevented the activation of AC activity by βAR and Gαs, it has no influence on the prostanoid receptors, which are located outside of caveolin-rich microdomains and can still activate AC. Both native cardiac myocytes and pulmonary artery endothelial cells overexpressing AC6 are inhibited by the NO donor S-nitroso-N-acetylpenicillamine (SNAP), inhibiting both basal and FSK-stimulated cAMP production (Ostrom et al., 2004). This process is subject to reversal by reducing agents, indicating the involvement of cysteine residue(s) as the target for S-nitrosylation (McVey et al., 1999; Ostrom et al., 2004). The reaction between NO and superoxide (O_2^-) results in the production of reactive nitrogen oxygen species (RNOS) capable of altering a broader variety of biomolecules than NO itself (Ridnour et al., 2004). At higher levels of O_2^-, NO inactivation is followed in turn by generation of the potent and short-lived oxidant peroxynitrite ($ONOO^-$/ONOOH), which can directly react with metal ions and thiols (Piacenza et al., 2022). $ONOO^-$ contributes more to S-nitrosylation of adjacent proteins than does NO (Maccarrone et al., 2000). It is also proposed that inhibition of AC by NO may be through S-nitrosylation caused by the reaction of another NO intermediate, nitrosonium (NO+), with cysteine residues (Tao et al., 1998). In a quantitative mass spectrometry screening investigation of modified cysteines utilizing a bioorthogonal cleavable-linker switch technique, AC6 featured among proteins identified as S-nitrosylated (Mnatsakanyan et al., 2019). AC6 inhibition due to S-nitrosylation was also demonstrated in pulmonary arteries (Sikarwar et al., 2018). Identification of AC6 cysteines susceptible to S-nitrosylation has been explored using site-directed mutagenesis of cysteines identified by bioinformatics analysis to reside within an SNO motif (Jaggupilli et al., 2018; Bhagirath et al., 2022). Mutation of cysteine 1004, located in the conserved C2 domain of AC6 near the Gαs docking position (Figure 6), decreases the basal and stimulated activity of AC6, indicating the importance of this residue in AC6 for intact catalytic activity and also susceptibility to inhibition if nitrosylated (Bhagirath et al., 2022).

AC5/6 regulation by other PTMs

AC6 may be phosphorylated through receptor tyrosine kinases (RTKs); Ser residues 603, 608, 744, 746, 750, and 754 have been implicated following RTK activation by IGF-1 or tyrosine phosphatase inhibition with sodium orthovanadate (Figure 6). Augmentation of AC6 catalytic function following RTK activity is inhibited by endogenous $p74^{raf-1}$ activity, but not by inhibitors of ERK, PKC, PKA, or PI3 kinase activity (Tan et al., 2001).

Role of AC5 in cardiovascular function

In this section, we review the studies reporting the role of AC5 in cardiomyopathies induced by catecholamine stress including chronic isoproterenol stimulation, aging, and pressure overload. Upregulated AC5 mRNA expression in spontaneously hypertensive rats suggests complex regulation of LV hypertrophy in hypertension (Vatner et al., 2013). Myocardial AC5 mRNA increases from 5 to 12 weeks in spontaneously hypertensive rats, associated with the development of LV hypertrophy (Fujino et al., 2003). On the other hand, AC5KO mice have diminished sympathetic and parasympathetic responses and disrupted Ca^{2+}-mediated cardiac regulation. Both basal and isoproterenol-stimulated AC activity

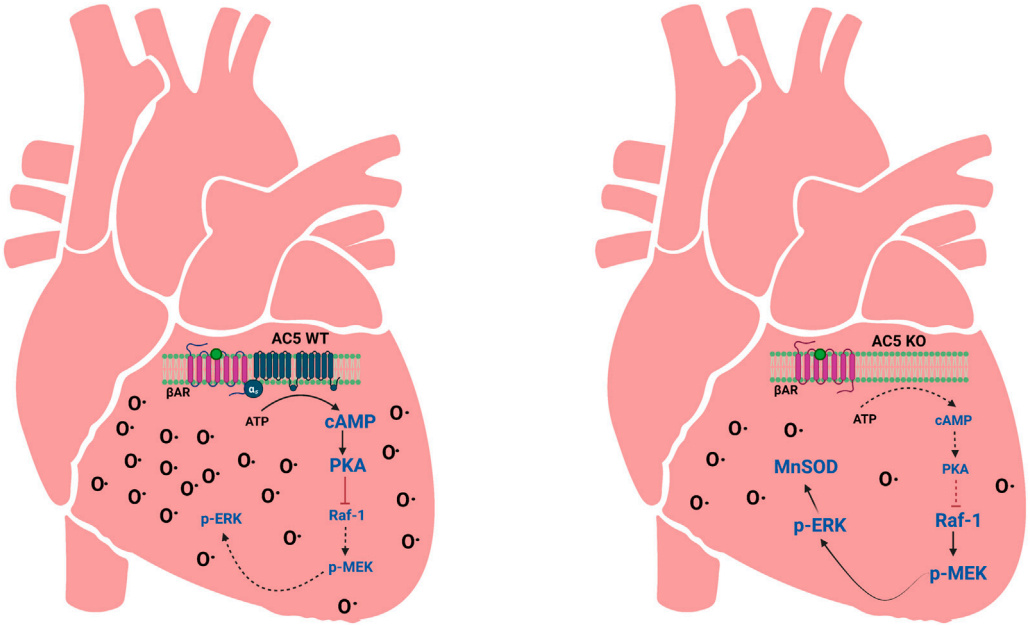

FIGURE 7
Depiction of AC5 signaling effects on cellular antioxidant imbalances in AC5WT and AC5KO hearts. When AC5 is disrupted (AC5KO), there is less βAR-stimulated cAMP, which decreases PKA activity. Lack of PKA leads to the activation of the Raf-1/MEK-ERK signaling pathway to increase the expression of MnSOD. This results in attenuation of oxidative stress. MnSOD antioxidant defense is not as present in AC5WT. O● indicates oxidative stress. Adapted from (Chester and Watts, 2007); image designed using BioRender.

are attenuated at 30%–40% in the cardiac membranes of AC5KO mice, with no compensatory increase in other AC isoforms; this reduction in AC activity does not alter cardiac function at baseline but compromises the LV inotropic response to adrenergic stimulation (Okumura et al., 2003a). In pressure overload, while there was no difference between AC5WT and AC5KO in cardiac muscle mass at baseline, AC5KO protected the heart from deleterious effects of pressure overload on LV ejection fraction, through restriction of myocardial apoptosis via upregulation of BCl-2 (Okumura et al., 2003b). Others have demonstrated enhancement in basal LV function in AC5KO as well as impairment in the responsiveness of LV to βAR stimulation (Tang et al., 2006). AC5KO mice also showed better heart function following chronic catecholamine stimulation, again through reduction in cardiac apoptosis, due to increased Bcl-2 expression and Akt signaling (Okumura et al., 2007). In contrast to the outcomes after prolonged isoproterenol infusion in AC5WT and AC5KO mice, the ejection fraction response of the LV to an abrupt isoproterenol challenge was lowered in AC5KO, which is in line with the downregulation of AC5 catalytic activity. These data indicate that AC5 impairs survival signaling after long-term catecholamine infusion, while AC5 deletion improves cardiac desensitization, suggesting a novel strategy for heart failure therapy.

Zhang et al. investigated ways in which selected Gαs-coupled receptors (GsPCRs) regulate cardiomyocyte viability by generating distinct signaling complexes governing pro-survival *versus* pro-death signaling. It was revealed that among the five GsPCRs studied, stimulation of β1AR and histamine-H2-receptor (H2R) has negative effects on the survival of cardiomyocytes, mediated through cAMP production by AC5 but not AC6 and via PKA activity stimulating pannexin-1 to release ATP into the extracellular space. On the other hand, activation of pro-survival GsPCRs adenosine-A2-receptor (A2R), calcitonin-gene-related-peptide-receptor (CGRPR), or relaxin-family peptide-receptor 1 (RXFP1) results in protective effects on cardiomyocyte survival as a function of cAMP generation by AC6, resulting in activation of cAMP efflux pumps. These findings indicate that selection of AC5 *versus* AC6 by GsPCRs determines cAMP localization which controls cAMP fate, thus altering cardiomyocyte survival (Zhang et al., 2023).

In an examination of heart rate variability during transient microgravity in parabolic flight, autonomic dysregulation became worse in AC5KO mice, while heart rate stability improved as a function of AC5 overexpression, indicating that AC5 may improve autonomic regulation (Okumura et al., 2008). It was further shown that AC5 activity is required to achieve constant responses in the low- and high-frequency ratio or normalized high frequency, two markers of sympathetic and parasympathetic activity, respectively (Bai et al., 2012). Thus, while inhibition of AC5 is beneficial for preventing cardiac myocyte myocardial apoptosis induced by excessive βAR stimulation, activation of this isoform may be advantageous in acute heart failure with low rate.

Transgenic mice overexpressing both AC5 and Gαq show efficient βAR-stimulated AC activity and cardiac contractility, but their hearts also appear pathologically fibrotic and hypertrophic (Tepe and Liggett, 1999). Microarray analysis of these hearts showed upregulation of various genes involved in pressure overload LV hypertrophy (Park et al., 2011). Examining genes relevant to ventricular hypertrophy upregulated in AC5 transgenic hearts even at baseline, transcription factor binding analysis revealed enrichment for the binding sites of nuclear factor of activated

TABLE 1 Physiological and pathophysiological effects of AC5 and AC6.

Study of cardiomyopathy	Physiological and pathophysiological effects			
	AC5	Ref	AC6	Ref
Chronic pressure-overload	$AC5^{-/-}$ showed better toleration, increased LV function, and less fibrosis and protected from apoptosis	Okumura et al. (2003b)	AC6-Tg (1) increased LV systolic wall stress, (2) reduced LVEF, and (3) unpreserved cardiac function	Guellich et al. (2010)
	AC5-Tg (1) manifested various upregulated genes related to LV hypertrophy and (2) marked enrichment of NFAT binding sites	Park et al. (2011)	Activation of AC6 expression led to better Ca^{2+} handling due to enhanced PLN phosphorylation, decreased NCX1 and PP1 expression, and increased SR $[Ca^{2+}]$	Sugano et al. (2011)
	Expression of AC5 but not AC6 is upregulated in AMPKα2 KO-TAC mice, leading to cause hypertrophy and aggravating of cardiac function, as evidenced by a higher decrease in EF	Garnier et al. (2023)	AC6KO resulted in reduced LV hypertrophy with no sign of LV dilation and protection of LV function in female mice. This was further correlated with reduced expression of FHL1 and periostin	Tang et al. (2010)
Longevity	AC5KO did not change in exercise performance. Increase in exercise capacity in AC5KO mice results in enhanced skeletal muscle function, not cardiac function	Vatner et al. (2015)	Cardiac-directed expression of AC6 protects the heart from myocardial hypertrophy, improves cardiac function, increases cAMP generation, and prolongs survival in Gαq cardiomyopathy	Roth et al. (2002)
Chronic catecholamine stress	MnSOD expression was downregulated, and its effect was 40% lower in AC5-Tg than in WT, but 40% higher in AC5KO, leading to cause chronic catecholamine stress	Lai et al. (2009)	$AC6^{\Delta N/\Delta N}$ and $AC6^{-/-}$ mice (1) responded more to apoptotic myocytes and cardiac remodeling. (2) AC6 plays the main role on Src-dependent STAT3 activation in the sarcolemma and preserved cardiomyocytes against cardiac stress through a PKA/STAT3-dependent pathway	Wu et al. (2017)

T-cells (NFAT), vital for the development and progress of cardiomyocyte hypertrophy (Park et al., 2011). NFAT binding determines the expression of cytoskeletal proteins (Schubert et al., 2003). So AC5 overexpression can mediate calcineurin–NFAT signaling involved in the development of LV hypertrophy (Park et al., 2011).

AC5KO mice also demonstrate increased physical performance, mediated through upregulation of the sirtuin-1 (SIRT1) pathway and regulating the antioxidant enzyme manganese superoxide dismutase (MnSOD) in the heart and liver of ACKO mice (Yan et al., 2012). It is proposed that SIRT1 is inhibited by AC5, leading to disruption of an interaction between SIRT1 and forkhead box O3 (FoxO3a), which eventually decreases MnSOD expression, thereby augmenting oxidative stress. The increase in MnSOD in myocytes subject to adenoviral AC5 KO is abolished by inhibition of either MEK or sirtuin, indicating MnSOD upregulation by both the mitogen-activated protein kinase kinase/extracellular signal-regulated kinases (MEK/ERK) and SIRT1/FoxO3a pathways (Chester and Watts, 2007; Lai et al., 2013). AC5KO mice are found to be resistant to cardiac stress with an enhanced median life span of roughly 30%, with protection from bone demineralization, and decreased susceptibility to fractures or aging-induced cardiomyopathy (Yan et al., 2007). The longevity, healthful aging, and stress resistance detected in AC5KO mice were correlated to diminished cAMP and PKA, resulting in activation of the Raf/MEK/ERK pathway, leading to the enhanced level of MnSOD (Yan et al., 2007). Additionally, AC5KO appears to increase NO signaling, demonstrating another mechanism by which AC5 may antagonize beneficial pathways induced by exercise (Guers et al., 2017). Two highly expressed genes encoding glutathione S-transferase (Gstk1 and Gstm) were downregulated in hypertrophic hearts, importantly because glutathione S-transferase plays the role of an antioxidant by conjugating glutathione on various substrates to protect the heart from oxidative stress (Cho et al., 2003; Yan et al., 2012).

Overall, these data place AC5 in a crucial role regulating life span and cardiac stress resistance. Figure 7 summarizes AC5 signaling in the context of cellular antioxidant imbalances in AC5WT and AC5KO. Studies delineating the role of AC5 in cardiovascular function are highlighted in Table 1.

Role of AC6 in cardiovascular function

In contrast to AC5, several studies have shown that increase in AC6 expression is beneficial for the failing heart, preserving LV contractile function and reducing dilation and dysfunction in hearts showing pressure overload (Takahashi et al., 2006; Sugano et al., 2011). The protective effect of AC6 in the heart is postulated as cAMP pathway dependence and independence (Tang et al., 2004; Gao et al., 2008; Gao et al., 2009; Gao et al., 2017). AC6 overexpression prevents cardiac hypertrophy, fibrosis, and cardiomyopathy (Roth et al., 1999; Tang et al., 2013), while cardiac-directed expression of catalytically inactive AC6 restored the detrimental effects of sustained catecholamine infusion, through diminished myocardial cAMP production (Gao et al., 2017). In addition, in ischemic cardiomyopathy, cAMP production and systolic and diastolic LV function are enhanced after activation of AC6 expression (Lai et al., 2008). AC6KO mice exhibit reduced cAMP production and have significantly higher mortality compared to AC6WT, but remain susceptible to βAR stimulation-induced cardiomyopathy, compromised electrophysiological characteristics including diminished longitudinal conduction velocity, and impaired connexin 43 phosphorylation at Ser 368, which may be part of the mechanism of ventricular dysfunction (Tang et al., 2013). Cardiac-directed AC6 overexpression with Gαq expression triggered

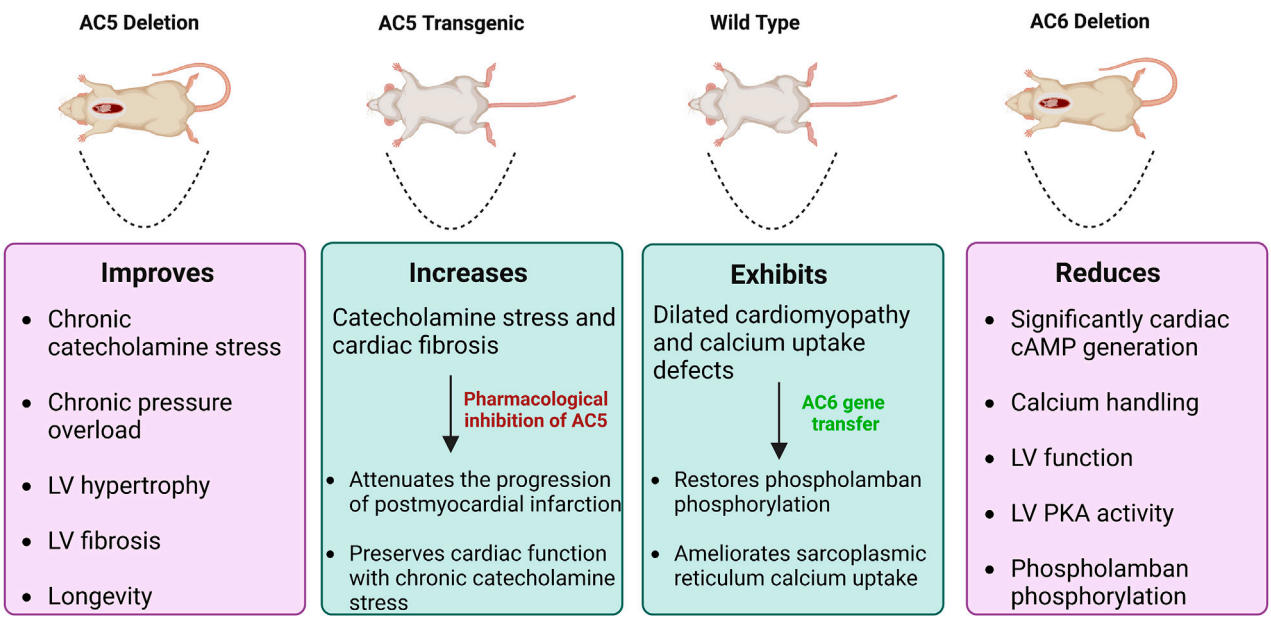

FIGURE 8
Effects of AC5 and AC6 overexpression or deletion on heart diseases *in vivo*. WT and transgenic mice subject to heart failure by catecholamine stress. Mice with deletion of AC5 demonstrate beneficial effects, whereas AC6 deletion is deleterious. AC5 transgenic mice have increased catecholamine stress and cardiac fibrosis. AC5 inhibitors rescue this condition, decreasing the progression of heart disease. Dilated cardiomyopathy in WT mice can be recovered by AC6 gene transfer.

improved βAR-stimulated AC activity, cAMP generation, and cardiac function *in vivo* and *ex vivo,* with no sign of hypertrophy and fibrosis (Roth et al., 1999), as well as improved Ca^{2+} handling and LV contractility manifested by ejection fraction, pressure development rate, and slope of the LV end-systolic pressure–volume relationship in aging mice (Tang et al., 2011). In 23-month-old rats, AC6 expression improved SR Ca^{2+} storage, while AC6 expression in 7-month-old mice did not show any difference in LV function and Ca^{2+} uptake (Tang et al., 2011). While cardiac troponin I (cTnI) phosphorylation diminishes with cardiac age (Jiang et al., 1993), improved LV function by AC6 is associated with phosphorylation of cTnI at Ser 23/24, which regulates thin filament function and thus contractility (Lai et al., 2008; Tang et al., 2011). While AC6 expression increases PKA activity and SR Ca^{2+} uptake (Tang et al., 2011), deletion of AC6 results in diminished PKA activity, phospholamban (PLB) phosphorylation, and decreased SR Ca^{+2}-ATPase activity 2a (SERCA2) affinity toward Ca^{+2} in failing hearts (Takahashi et al., 2006; Tang et al., 2008). Increased AC6 content increases the expression of activating transcription factor-3, which extinguishes PLB promoter activity, resulting in reduced PLB expression (Gao et al., 2004).

Enhanced AC6 expression is also correlated with increased nuclear phospho-Akt promoting phosphorylation of Akt at Ser473 and Thr308. This process appears to be independent of PKA or βAR stimulation (Gao et al., 2008). Akt activity is reversely regulated by PH domain leucine-rich repeat protein phosphatase (PHLPP), responsible for dephosphorylation of Akt at Ser473. AC6 inhibits PHLPP activity in cardiomyocytes, bringing about high levels of Akt phosphorylation. This PHLPP suppression is, however, rescued rapidly by isoproterenol and FSK stimulation, leading to significant dephosphorylation of Akt at Ser473, but not Thr308. As PLB is an Akt target, active phospho-Akt increases PLB activity and thus improves sarcoplasmic Ca^{2+} cycling (Gao et al., 2009). Figure 8 gives an illustrated summary of the effects of AC5 and AC6 addition or deletion *in vivo*.

As AC6 shows remarkable physiological benefits for the heart, while its content and function are diminished in the failing heart (Ostrom et al., 2022), there has been increased interest in the efficacy and safety of adenoviral delivery of AC6 in patients with heart failure (Hammond et al., 2016) (AC6 Clinical Trials). In a mouse model, the AC6 C_1–C_2 construct was shown to effectively improve cardiac dysfunction caused by prolonged βAR stimulation (Roth et al., 2002). Although AC6 transgenic mice with cardiac-directed C_1–C_2 expression exhibited less cAMP production, they preserved normal cardiac function through ameliorated Ca^{2+} handling and tolerated sustained isoproterenol infusion and pressure overload without detrimental effects on LV contractility (Gao et al., 2016; Tan et al., 2019). Preclinical analysis of AC6 gene therapy recently transitioned into clinical trials. The phase 2 results for one-time AC6 gene transfer in 56 adult patients with symptomatic heart failure (ischemic or non-ischemic) and an ejection fraction of 40% resulted in safely ameliorated LV function above standard heart failure therapy (Hammond et al., 2016). The subsequent FLOURISH trial, a double-blinded placebo-controlled, multicenter phase 3 trial of 536 patients, aims to decrease heart failure hospitalization rates and improve ejection fraction, while minimizing adverse events after intracoronary injection of the human adenovirus 5 encoding human AC6 (Ad5. hAC6) gene in patients with heart failure and diminished LV ejection fraction; patient recruitment is ongoing (Penny et al., 2018).

Studies contextualizing the AC6 role in cardiovascular function are highlighted in Table 1.

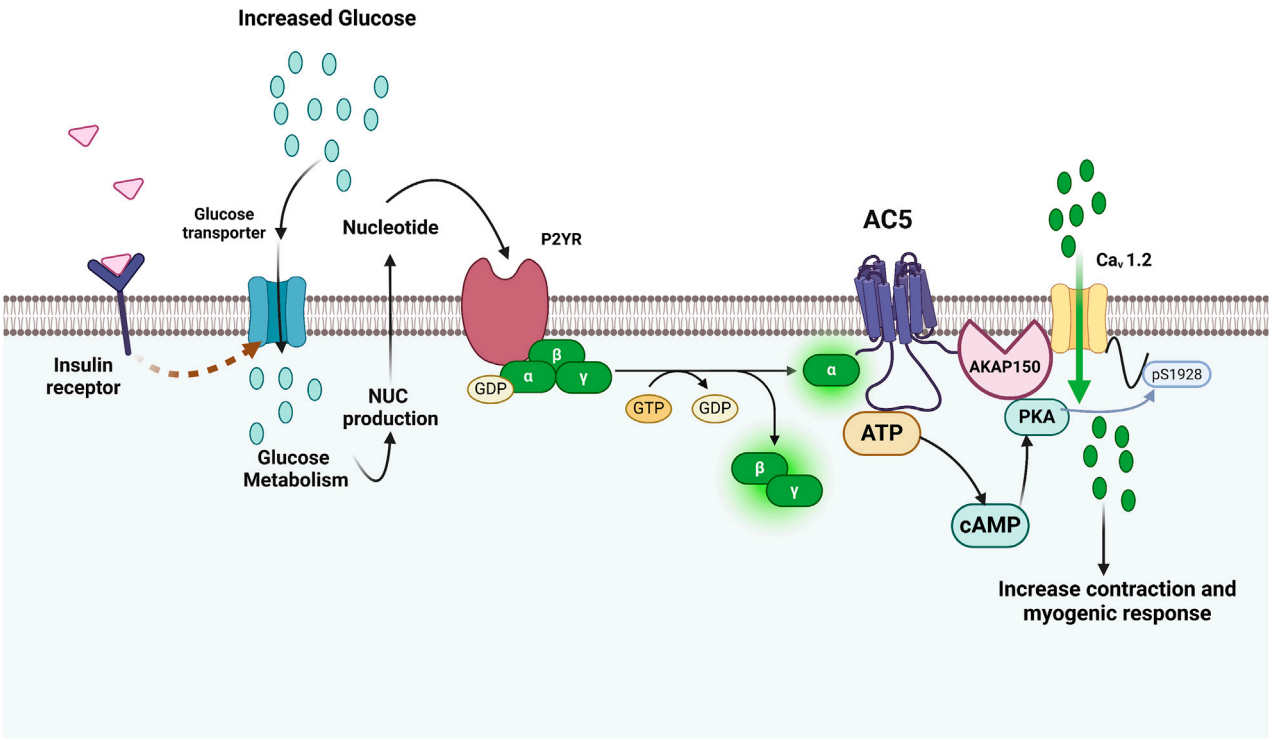

FIGURE 9
Involvement of AC5-mediated localized cAMP generation in diabetes and extracellular glucose activation of L-type Ca2+ channels and vasoconstriction. The illustration shows how glucose regulates L-type Ca^{2+} channel activity and vascular reactivity in an AC5-dependent manner. Increases in extracellular glucose can trigger P2YR linked to Gαs signaling through extracellular nucleotide signaling because of transport and metabolism. Because AC5 and $Ca_V1.2$ are close in proximity, this cAMP microdomain may stimulate a pool of AKAP150-anchored PKA that is jointly linked to $Ca_V1.2$, making it to become more phosphorylated at Ser1928 and increasing channel function. Adapted from (Syed et al., 2019); image designed using BioRender.

Roles of AC5 and AC6 in vasculature

AC activity in vascular cells regulates vascular reactivity, apoptosis, hypertrophy, and proliferation (Gros et al., 2006). The distribution of AC isoforms varies in the vasculature compared to the myocardium. Based on RT-PCR studies, ACs 3, 5, and 6 are expressed in the adult rat aorta (Ostrom et al., 2002), while in the perinatal period, the ductus arteriosus expresses more AC2 and 6 than does the aorta (Yokoyama et al., 2010). The pulmonary artery expresses AC6 primarily, followed by ACs 7, 9, and 3 (Sikarwar et al., 2018). In vascular myocytes, AC6 is the main AC isoform involved in βAR-mediated cAMP/PKA signaling and activation of the K_{ATP} current, important for harmonizing the membrane potential and regulating vascular tone (Nelson et al., 2011). Subjects who carry the genetic variation *ADCY6 A674S* have increased blood pressure, with a hyperdynamic cardiac profile that is compatible with the effect of elevated AC function (Hodges et al., 2010). Vasodilation is antagonized by Gαq-mediated signaling, in part by direct Ca^{2+} inhibition of AC5 and AC6 (von Hayn et al., 2010).

Changes in AC activity have been related to the development of diabetes, heart failure, and hypertension (Matsumoto et al., 2005; Hodges et al., 2010). Abnormal arterial myocyte contractility, in addition to compromised endothelium-dependent vasodilation, is an important contributing factor to vascular complications including altered myogenic tone, both in diabetic mice and in patients with diabetes (Montero et al., 2013; Sena et al., 2013; Tykocki et al., 2017; Syed et al., 2019). The contractile state of smooth muscle cells in the vessel wall determines the vascular tone, measured by a balance between the effects of vasoconstrictor and vasodilator signaling pathways, inclusive of adrenergic receptors and ACs (Shi et al., 2020). Physiological targets of ACs and downstream PKA include potassium channel phosphorylation, inducing hyperpolarization and vasodilation (Nelson et al., 2011). In resistance arteries and arterioles, the myogenic tone generated through the pulsatile stretch of the vascular wall modulates baseline smooth muscle contraction (Tykocki et al., 2017); in diabetic hyperglycemia, altered expression or function of potassium channels is linked to increased myogenic tone (Syed et al., 2019). Elevated glucose can trigger Gαs signaling (Lemaire et al., 2004) but may drive vasoconstriction through glucose-induced cAMP production via AC5, which results in the activation of an anchored PKA pool, and in turn, phosphorylates the L-type Ca^{2+} channel pore-forming $Ca_V1.2$ subunit at Ser 1928 (Nystoriak et al., 2017; Prada et al., 2019; Syed et al., 2019). These interactions cause potentiation of L-type Ca^{2+} channel activity, enhanced $[Ca^{2+}]_i$, and vasoconstriction (Figure 9). Supporting this, interruption of AKAP5 function in arterial myocytes prevents cAMP generation in response to either increased glucose or selective purinergic P2Y11 agonist NF546; in AKAP5-null arterial myocytes or arteries, there is no clustering of P2Y11/P2Y11-like receptors,

AC5, PKA, and $Ca_V1.2$ into nanocomplexes at the plasma membrane; therefore, glucose- and NF546-induced potentiation of L-type Ca^{2+} channels and vasoconstriction does not occur (Prada et al., 2020). These data implicate AKAP5 and AC5 in the spatial confinement of cAMP signaling induced by elevated glucose via activation of P2Y11/P2Y11-like receptors in arterial myocytes (Prada et al., 2019; Prada et al., 2020).

ACs in endothelial cells play a role in vascular permeability. Prostacyclin-mediated signaling via AC6 (but not AC5) forms part of a feedback circuit that increases endothelial barrier function. Endothelial cells overexpressing AC6 have increased prostacyclin response, reducing the permeability of the endothelial barrier. In human umbilical vein endothelial cells, adenoviral-mediated gene transfer of AC6 increased prostacyclin receptor-stimulated cAMP synthesis and concurrently decreased thrombin-stimulated increases in endothelial cell barrier function (Bundey and Insel, 2006).

Role of ACs in cardiac automaticity

The sinoatrial node (SAN) is a crescent-like shaped cluster of myocytes split by connective tissue, spread over a few millimeters (Kashou et al., 2017), located at the convergence of the superior vena cava opening and the crista terminalis in the upper wall of the right atrium. The SAN comprises coordinated actions of pacemaker cells capable of generating a cyclic electrical impulse (Kashou et al., 2017). Cardiac arrhythmia due to the abnormality of the SAN can affect annually up to 1 per 1000 adults >45 years of age (John and Kumar, 2016). The SAN is the main regulator of heart rate and is regulated by βAR signaling (Irisawa et al., 1993; Tsutsui et al., 2021). While the expression of a number of AC isoforms (types 1, 2, 3, 5, 6, 8, and 9) has been identified in rabbit SAN, AC1 and AC8, two of the Ca^{2+}-activated AC isoforms, are predominantly distributed in atrial and SAN cells (Mattick et al., 2007; Younes et al., 2008; Robinson et al., 2021). In contrast, a recent study showed AC1 and AC6 but not AC8 expressed in SAN cells at the transcript and protein levels (Ren et al., 2022).

Ca^{2+} is a vital modulator of pacemaker potential via the Ca^{2+} clock (Maltsev and Lakatta, 2012), where the ryanodine receptor 2 (RyR2) facilitates the spontaneous release of Ca^{2+} from the SR, which in turn pushes Ca^{2+} to be released from the cytosol via the Na^+–Ca^{2+} exchanger (Maltsev and Lakatta, 2012; Ren et al., 2022). Adrenergic control of the cardiac pacemaker current has been ascribed to AC1 (Mattick et al., 2007). AC1 mediates cAMP signaling in the SAN, in a functional microdomain with CAV3, hyperpolarization-activated cyclic nucleotide-gated 4 (HCN4), $Ca_v1.2$, and RyR2. cAMP released by AC1 leads to elevation of intracellular Ca^{2+} via Ca^{2+} channels, which triggers a positive feedback to AC1 and negative feedback to AC5/6 (Ren et al., 2022). While cardiac-specific overexpression of AC8 transgenic mice in the SAN is reported to augment the heart rate and rhythm (Moen et al., 2019), others found no differences in automaticity, basal heart rate, or isoproterenol responses in AC8-null mice compared with wild-type (Ren et al., 2022). In guinea pig atrial myocytes, sarcoplasmic reticulum type 2 inositol trisphosphate (IP3) receptors colocalize with AC8, with AC1 localized proximally. Functional AC1 and AC8 are required for the positive chronotropic effect of phenylephrine on the SAN, and activity of both AC1 and AC8 plus PKA is required for the effect of IP3 on cellular Ca^{2+} transients (Capel et al., 2021).

Pacemaker current is generated by hyperpolarization-activated cyclic nucleotide-gated channels (HCN). To assess the role of Ca^{2+} homeostasis in autonomic regulation, AC1 and AC6 were expressed in cultures of spontaneously beating neonatal rat ventricle cells co-expressing HCN2. AC1, but not AC6 expression, increased intracellular cAMP and automaticity; AC1-mediated cAMP generation was resistant to β-adrenergic blockade, but the HCN2 response to an adrenergic agonist in the presence of AC1 (but not AC6) was sensitive to Ca^{2+} chelation, implying that the effect of Ca^{2+} homeostasis on the adrenergic regulation of the pacemaker rate could be accounted by the existence of a Ca^{2+} sensitive AC isoform (Kryukova et al., 2012; Robinson et al., 2021). However in a study of *in vivo* adenoviral gene transfer of AC6 in a porcine atrioventricular node block model, the AC6 injected group developed an escape rhythm of ~100 beats/min originating at the LV injection site, while control animals had RV escape rhythms, suggesting that biological pacemaker activity could also be triggered by AC6 (Ruhparwar et al., 2010).

AC5 and AC6 as potential drug targets for cardiovascular disease

Although βAR agonists and antagonists have therapeutic effectiveness for heart failure treatment, there are still patients who do not respond effectively; thus, heart failure is the most common cause of mortality worldwide (Capote et al., 2015). A weak cardiac response to catecholamine stimulation is a characteristic of the heart failure phenotype; hence, stimulation of the adrenergic pathway has been targeted to increase cardiac function. However, βARs can undergo downregulation after prolonged stimulation by agonists or antagonists, which leads to a reduction in their cell surface density (tachyphylaxis), modifications in subtype composition, or increase in PKA or G-protein-coupled receptor kinase (GRK) activity, resulting in uncoupling of βAR from G proteins (desensitization) (Mahmood et al., 2022). βAR desensitization also serves as a compensatory mechanism, which may disrupt cardiac function and promote arrhythmias (Ho et al., 2010).

In contrast to GPCRs, AC5 and AC6 are less likely subject to desensitization after sustained ligand exposure (Pierre et al., 2009). The AC5 and AC6 isoforms have been indirectly targeted by GPCR agonists or antagonists and PDE inhibitors due to their action as the central relay site that assembles and amplifies a wide variety of signals (Pavan et al., 2009). AC5 and AC6 have pivotal roles in cardiac disease. Despite the promising results from knockout and transgenic mice, suggesting that AC5 and AC6 can be potential drug targets, advancements to selectively and therapeutically target these isoforms have been hindered by their structural similarity. However, many positive steps in this direction are reviewed below.

AC5 and AC6 activators for the treatment of heart failure

The most potent stimulus for increasing the cardiac output is initiated by sympathetic nervous system activation through the

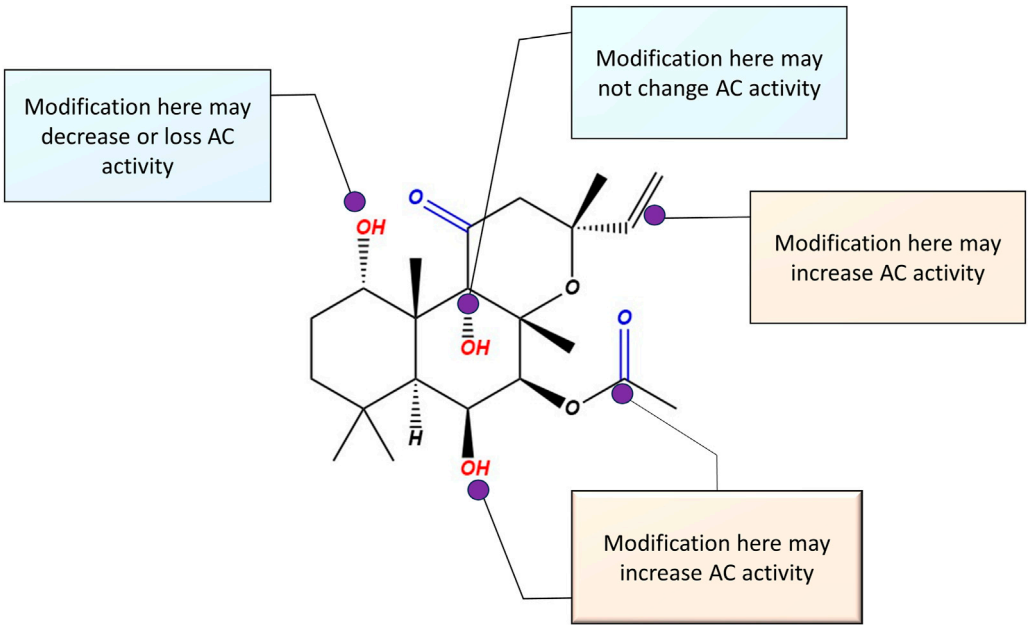

FIGURE 10
Possible modification sites on the parent compound forskolin molecule, for the design of selective AC5 or AC6 activators. Based on previous studies, any modification on C (1) reduces AC activity. Modifications on C (6) and (7) have the potential to enhance AC5 and AC6 activity.

activation of βAR and Gαs, which in turn activates AC5 or AC6 to enhance cardiac contractility and rate. In the failing heart, production of basal and stimulated cAMP is diminished, but driving cardiac cAMP levels via adrenergic agents can result in higher long-term mortality (Bristow et al., 1982; Packer et al., 1991). FSK is a well-known activator of all transmembrane ACs, but its lack of selectivity impedes its clinical application; in addition to ACs, it also targets nuclear receptors, ion channels, glucose transporters, and P-glycoprotein multidrug resistance transporters (Dessauer et al., 2017). Clinically, in patients with heart failure, forskolin decreases ventricular filling pressures and vascular resistance while increasing cardiac output, ejection fraction, and stroke volume (Bristow et al., 1984). However, novel FSK-derived compounds have been synthesized that selectively target AC5 and AC6 isoforms (Pavan et al., 2009).

To design selective AC5 and AC6 activators, maintaining hydrogen bonding of FSK C1-OH, C7-acetyl, and C9-OH has been deemed crucial (Onda et al., 2001). There has also been specific focus on modification of C6 and C7 positions, due to a large open area apparent from AC crystallographic studies (Tesmer et al., 1997), suggesting that modification on these FSK positions may increase AC activity with selectivity and/or specificity. Figure 10 illustrates the possible sites of modifications for designing novel AC5 or AC6 activators. It is important to note that many of the earlier studies which established FSK positions suitable for modification evaluated only a subset of the AC isoforms. Testing of candidate compounds on each AC isoform remains necessary to determine which site is particular to each isoform.

The development of AC5 activator 6-[3-(dimethylamino) propionyl]FSK (NKH477, FD5, or colforsin daropate hydrochloride) (Toya et al., 1998) grabbed the attention of scientists working on AC drug discovery. Colforsin is a potent water-soluble derivative of the C6 position of FSK, initially approved in Japan for the treatment of advanced congestive heart failure due to the elevation of cAMP in cardiac tissue, resulting in the enhancement of cardiac contractility. From the standpoint of AC isoform selectivity, colforsin activates AC5 > AC2~AC3 (Toya et al., 1998). Using the isolated perfused canine heart, the chronotropic, inotropic, coronary vasodilatory effects, and AC activity of colforsin were compared to those in catecholamines isoproterenol, dopamine, and dobutamine. All the drugs demonstrated positive chronotropic, inotropic, and coronary vasodilator effects. Colforsin cardiovascular actions were in the following order: coronary vasodilation >> positive inotropy > positive chronotropy, while isoproterenol, dopamine, and dobutamine evinced positive inotropy >> coronary vasodilation > positive chronotropy. While inotropic effects are desirable, chronotropic agents have potential arrhythmogenic effects and may impair cardiac filling; however, at doses with comparable inotropic effects, colforsin demonstrated a higher positive chronotropic effect than catecholamines or PDE inhibitors, with ventricular tachycardia triggered equally by colforsin and isoproterenol. Nevertheless, the coronary vasodilator activity of colforsin was more potent. The strong coronary vasodilator function of colforsin may therefore be applicable for the treatment of heart disease where coronary blood flow is limited and βAR-dependent signaling is also downregulated (Yoneyama et al., 2002). In a model of canine respiratory acidosis causing cardiac dysfunction, colforsin increased cardiac rate and decreased systemic vascular resistance to the same degree as did dobutamine, without attenuation by acidosis (Itami et al., 2019). Colforsin has also demonstrated pulmonary artery vasodilation (Yokochi et al., 2010), which is mediated largely through AC6 rather than AC5 (Sikarwar et al., 2018), indicating that colforsin may not be AC5-selective (Jaggupilli

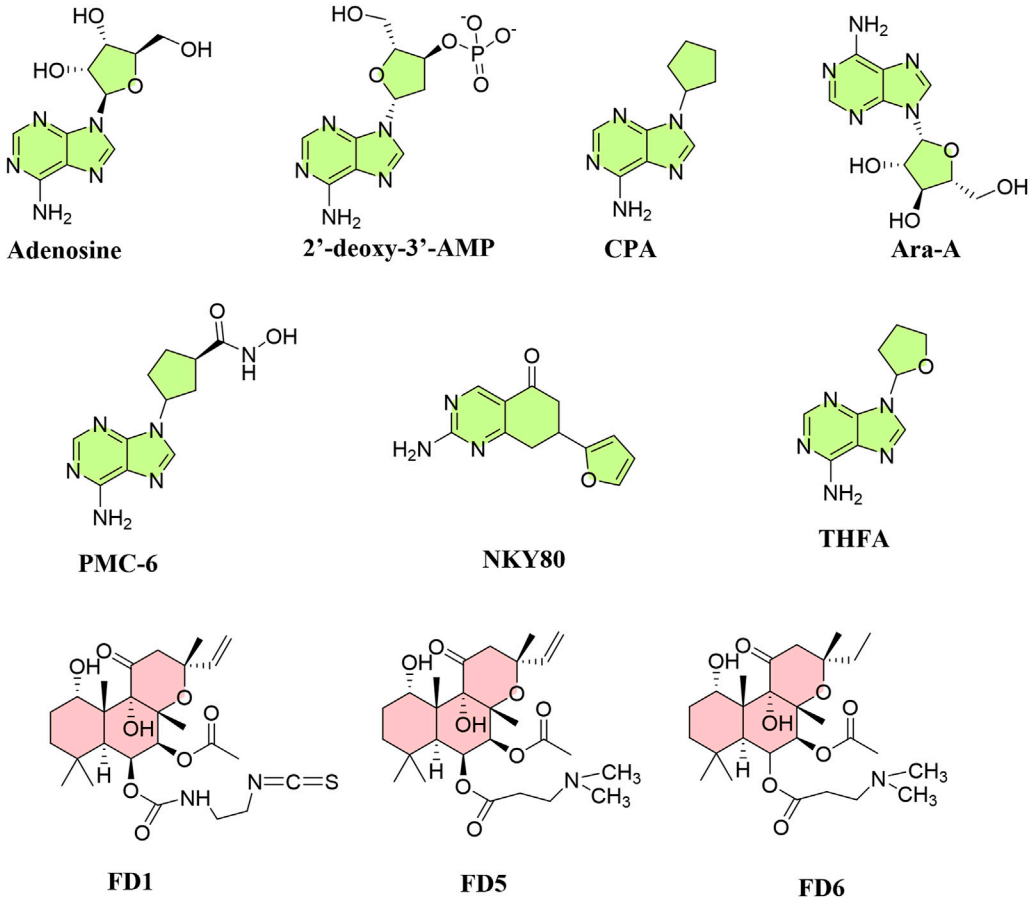

FIGURE 11
Structures of known inhibitors and activators targeting AC5 and AC6 allosteric or catalytic sites. Inhibitors (green) are mainly P-site inhibitors, and activators (red) are forskolin derivatives.

et al., 2018). 6-[3-(Dimethylamino)propionyl]-14–15-dihydro-FSK (FD6) is another FSK derivative, structurally similar to colforsin, but with reduced 14–15 alkyne bond, that showed AC5 and AC6 selectivity (Yokoyama et al., 2010). The lack of complete AC5 *versus* AC6 selectivity of colforsin and similar compounds does complicate our understanding of isoform-specific cardiac effects of AC activation, in the absence of more preclinical data. Protective or detrimental effects of individual AC manipulations on cardiac function can perhaps be best deciphered from gene overexpression or deletion studies; precise pharmacological interrogation of AC5 or AC6 activation would require further development of more selective tools.

A list of published inhibitors and FSK derivatives having AC5 and/or AC6 selectivity is depicted in Figure 11.

AC5 and AC6 inhibitors for the treatment of heart failure

As AC5 deletion appears to be protective in heart failure, there has been much research into isoform-selective AC5 inhibitors, which may be advantageous over β-blockers (Pierre et al., 2009). AC inhibitors are classified into two categories: competitive inhibitors [(M)ANT- and TNP-nucleotides] and non-competitive P-site inhibitors. Among AC5 and AC6 inhibitors discovered, 2′-d-3′-MANT-GDP has inhibitory effects on AC6 (~8-fold) compared to AC5 (Gille et al., 2004). Among P-site inhibitors with metal chelating characteristics (PMC), PMC-6 has shown selectivity for AC5, protecting cardiac myocytes from βAR-induced apoptosis without degrading cAMP synthesis or contractility (Iwatsubo et al., 2004). P-site inhibitors that are adenosine analogs interact with ACs when PP_i is present and act by obstructing the interaction of other substrates with P-sites (Dessauer and Gilman, 1997). Unlike FSK, P-site inhibitors attach to the catalytic site for substrate ATP (Tesmer et al., 2000), generating a dead-end complex with PPi (Bushfield et al., 1990). Ribose-substituted P-ligands such as 9-(cyclopentyl)-adenine (CPA) and 9-(tetrahydrofuryl)-adenine (THFA) have IC_{50} values in the micromolar range, selectively inhibiting AC5 more than AC3 and AC2; while 2′-deoxy-3′-AMP and 3′-AMP inhibit AC3 and AC5 more than AC2 (Onda et al., 2001; Iwatsubo et al., 2003). 2-Amino-7-(2-furanyl)-7,8-dihydro-5(6H)-quinazolinone (NKY80), derived from 9-(tetrahydro-2-furanyl)-9H-purin-6-amine (SQ22,536), like 9-(tetrahydro-2-furyl)adenine (THFA), inhibits AC5 despite not possessing an adenine ring. NKY80, though less potent, exhibits a similar AC5 selectivity to THFA in the inhibition of AC5 catalytic

activity, having a selectivity ratio of 210 between AC5 and AC2 with an IC50 of 8.3 μM for AC5, 132 μM for AC3, and 1.7 mM for AC2 when Gαs–GTPγS–forskolin is present (Onda et al., 2001).

The antiviral drug adenine 9-β-D-arabinofuranoside (Ara-A), known as vidarabine, has been found to selectively inhibit AC5; Ara-A markedly diminished AC activity in AC5 transgenic mice, but not in AC5KO, and had a minor effect in either WT or AC6 transgenic mice (Iwatsubo et al., 2012). However, another study indicated that Ara-A is also a potent AC6 inhibitor (pIC_{50}: 5.67 and 5.34, for AC5 and AC6, respectively); indeed, SQ22,536, NKY80, and Ara-A inhibit both AC5 and AC6 without distinguishing them (Brand et al., 2013). Inhibition of AC5 by Ara-A is thought to be mediated through the MEK/ERK pathway. The Ca^{2+}-binding protein annexin A4 (ANXA4) selectively inhibits AC5. Both ANXA4 and a peptide encompassing the ANXA4 N-terminal sequence ($A4N_{1\text{-}22}$) reduced cAMP generation in AC5; ANXA4 co-immunoprecipitates with AC5 but not AC6, binding to its N-terminal domain. The peptide $A4N_{1\text{-}22}$ diminishes recruitment of the L-type Ca^{2+} current (I_{CaL}) and prevents action potential prolongation after catecholamine challenge, an effect similar to the loss of the β1AR signal in AC5KO models (Heinick et al., 2020). Another novel AC5 inhibitor, C90, inhibits cAMP production to FSK by 42% in WT but not in AC5KO, suggesting its selective AC5 inhibitory effect; it is five times less potent in inhibiting AC2 and AC6. C90 also produced the interesting effect of decreasing myocardial infarct size even when administered after coronary reperfusion (Zhang et al., 2018).

Conclusion

Although there have been a number of AC5 and AC6 activators/inhibitors tested *in vitro*, more *in vivo* studies are required for evaluation of the cardiac AC isoforms as drug targets and more precision of selectivity testing, given the similar structures and expressions patterns of cardiac ACs. Advances in detailed structural information (Qi et al., 2019) as well as computational homology modeling and mutational analysis of ligand-binding regions (Bhatia et al., 2023) will provide further direction to drug discovery for selective targeting of cardiovascular ACs, as will the selection of appropriate pharmacokinetic features and targeted drug delivery systems to decrease off-target adverse effects.

The prevalence of cardiovascular disease is on the rise, despite the rapid advancements in drug discovery for therapeutics addressing the alteration in the adrenergic signaling profile in the failing heart. Increased activity of AC6 has a beneficial effect on cardiac contractility, cell survival, and Ca^{2+} handling, while the activation of AC5 is deleterious. Differentiating the regulation of AC5 and AC6 and their signaling pathways opens up potential avenues for selective manipulation of physiologically important cAMP pools. The development of AC5/6 selective activators or inhibitors is still in its infancy, generating novel stimulators and inhibitors or taking advantage of drug repositioning; AC6 gene transfer also holds promise for heart failure treatment. Further research in these directions is clearly warranted.

Author contributions

SM: conceptualization, data curation, supervision, and writing–original draft. RS: writing–original draft. BV: writing–original draft. SD: conceptualization, funding acquisition, project administration, resources, supervision, and writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Yusof Kamisah,
Faculty of Medicine Universiti Kebangaan Malaysia, Malaysia

REVIEWED BY
Wei Sun,
University of Pittsburgh Medical Center, United States
Dezhao Lu,
Zhejiang Chinese Medical University, China

*CORRESPONDENCE
Cheng Zeng,
zengcheng@gdpu.edu.cn

†These authors have contributed equally to this work

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NLRP3 inflammasome and pyroptosis in cardiovascular diseases and exercise intervention

Ping Ding[1†], Yuanming Song[1†], Yang Yang[2] and Cheng Zeng[1,3,4*]

[1]Center for Drug Research and Development, Guangdong Pharmaceutical University, Guangzhou, China, [2]Zhuhai People's Hospital, Zhuhai Clinical Medical College of Jinan University, Zhuhai, China, [3]Key Specialty of Clinical Pharmacy, The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, China, [4]Guangdong Key Laboratory of Pharmaceutical Bioactive Substances, Guangdong Pharmaceutical University, Guangzhou, China

NOD-like receptor protein 3 (NLRP3) inflammasome is an intracellular sensing protein complex that possesses NACHT, leucine-rich repeat, and pyrin domain, playing a crucial role in innate immunity. Activation of the NLRP3 inflammasome leads to the production of pro-inflammatory cellular contents, such as interleukin (IL)-1β and IL-18, and induction of inflammatory cell death known as pyroptosis, thereby amplifying or sustaining inflammation. While a balanced inflammatory response is beneficial for resolving damage and promoting tissue healing, excessive activation of the NLRP3 inflammasome and pyroptosis can have harmful effects. The involvement of the NLRP3 inflammasome has been observed in various cardiovascular diseases (CVD). Indeed, the NLRP3 inflammasome and its associated pyroptosis are closely linked to key cardiovascular risk factors including hyperlipidemia, diabetes, hypertension, obesity, and hyperhomocysteinemia. Exercise compared with medicine is a highly effective measure for both preventing and treating CVD. Interestingly, emerging evidence suggests that exercise improves CVD and inhibits the activity of NLRP3 inflammasome and pyroptosis. In this review, the activation mechanisms of the NLRP3 inflammasome and its pathogenic role in CVD are critically discussed. Importantly, the purpose is to emphasize the crucial role of exercise in managing CVD by suppressing NLRP3 inflammasome activity and proposes it as the foundation for developing novel treatment strategies.

KEYWORDS

cardiovascular disease, exercise, pyroptosis, NLRP3 inflammasome, intervation

1 Introduction

Cardiovascular diseases (CVD) remain a prevalent global health concern, causing a significant burden of illness and mortality, with approximately one-third of all deaths attributed to this condition (Mensah et al., 2019). The common symptoms of CVD include chest pain, shortness of breath, irregular heartbeat, fatigue, and decreased physical stamina (Tutor et al., 2023; Wong and Sattar, 2023). CVD encompass various disorders that affect the heart and blood vessels, including atherosclerosis (AS), obesity, diabetes, hyperhomocysteinemia (HHcy), myocardial infarction (MI), hypertension, heart failure (HF), and diabetic cardiomyopathy (DCM) (Konishi et al., 2022; Haidar and Horwich, 2023). The conventional risk factors that are widely recognized for CVD, such as hypertension, hypercholesteremia, diabetes mellitus, and cigarette smoking, remain

acknowledged as the main factors responsible for the development and advancement of this condition (Alten et al., 2020).

NOD-like receptor protein 3 (NLRP3) inflammasome is a molecular platform that triggers caspase-1 and facilitates the secretion of interleukin (IL)-1β and IL-18 in response to cellular infection or stress (Valenzuela et al., 2023). This activation results in the cleavage of gasdermin D (GSDMD) by caspase-1, generating an N-terminal GSDMD fragment (Valenzuela et al., 2023). This fragment induces the formation of membrane pores and triggers inflammatory cell death, namely pyroptosis (Valenzuela et al., 2023). The involvement of NLRP3 inflammasome and pyroptosis has been established in cardiovascular risk factors such as hyperlipidemia, diabetes, hypertension, obesity, and HHcy (Alten et al., 2020). Targeting NLRP3 inflammasome activation and pyroptosis holds great potential for therapeutic interventions against CVD.

Extensive research has demonstrated that exercise plays a crucial role in weight management (Murray et al., 2023), blood pressure (BP) reduction (Tucker et al., 2022), blood sugar (Kar et al., 2019) and lipid regulation (Lee et al., 2020), consequently lowering the risk of CVD (Li et al., 2023). Moreover, moderate exercise improves cardiovascular system function and structure, strengthens the heart muscle, enhances cardio-pulmonary function, promotes blood circulation, and increases the heart's tolerance and overall health (Alten et al., 2020). Furthermore, exercise exhibits a clear anti-inflammatory effect (Lee et al., 2020). It is widely acknowledged that exercise can effectively reduce chronic inflammation by inhibiting the expression of inflammatory factors while increasing the release of anti-inflammatory cytokines (Lee et al., 2020). Previous studies have indicated that exercise can decrease the activation of the NLRP3 inflammasome to substantially inhibit IL-1β, and IL-18 release (Li et al., 2023; Liu et al., 2023). This review mainly focuses on how exercise can improve CVD by influencing NLRP3 inflammasome or pyroptosis. Additionally, this review will investigate exercise as a therapeutic strategy via NLRP3 inflammasome for managing CVD and address the research questions that need to be explored in the future.1.

2 Overview of NLRP3 inflammasome

Inflammasomes are crucial components of the immune system that play a major role in initiating inflammatory responses (Chang, 2023). They are composed of sensor proteins known as pattern recognition receptors that oligomerize and form a platform for the activation of caspase-1 in response to damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) (Chang, 2023). The nucleotide-binding domain-like receptor (NLR) family all share a central nucleotide-binding domain, and most members have a C-terminal leucine-rich repeat (LRR) domain and a variable N-terminal domain (Swanson et al., 2019). While certain members such as NLRP1, NLRP3, and NLRC4 are recognized as NLRs capable of forming inflammasomes, others like NLRP6 and NLRP12 are considered potential inflammasome sensors (Swanson et al., 2019). The NLRP3 inflammasome, in particular, is essential for the host's immune defense against various bacterial, fungal, and viral infections (Dilucca et al., 2021). However, when dysregulated, it has been implicated in the development of several inflammatory disorders, including CVD (Paerewijck and Lamkanfi, 2022).

The NLRP3 inflammasome is composed of several key components. The NLR protein in the NLRP3 inflammasome contains a conserved nucleotide-binding and oligomerization domain, C-terminal LRRs, and a pyrin domain (PYD) that facilitates multimerization (Xi et al., 2024). Upon activation of the NLRP3 inflammasome, the NLRs oligomerize through their nucleotide-binding and oligomerization domains (Fu and Wu, 2023). This leads to the recruitment of the adaptor protein apoptosis-associated speck-like protein (ASC) through PYD-PYD interactions (Fu and Wu, 2023). ASC then forms large speck-like structures and recruits pro-caspase-1 through caspase recruitment domain (CARD)-CARD interactions. Pro-caspase-1 undergoes autocatalytic cleavage, resulting in the formation of active caspase-1 p10/p20 tetramers (Ruan, 2019). These active caspase-1 tetramers mediate the maturation and secretion of IL-1β and IL-18 (Ruan, 2019; Fu and Wu, 2023). Additionally, caspase-1 can cleave GSDMD to generate GSDMD n-terminal (NT). GSDMD-NT forms plasma membrane pores, leading to the induction of pyroptosis (Lee et al., 2021; Nie et al., 2021). The canonical activation of inflammasomes is proposed to occur in two steps: priming and assembly (Bockstiegel et al., 2023). Priming involves the initial activation of toll-like receptors by agonists such as lipopolysaccharide (LPS) (Fu and Wu, 2023). This trigger signaling cascades, primarily through the nuclear factor-κB (NF-κB) pathway, which leads to the transcriptional upregulation of pro-inflammatory mediators like pro-IL-1β. Following priming, the activated inflammasome assembles in response to various PAMPs or DAMPs (Fu and Wu, 2023; Krantz et al., 2023). This assembly forms a large molecular platform that activates inflammatory caspases and processes pro-IL-1β (Huang et al., 2023). The activation of NLRP3 inflammasome requires "priming" with TLR agonists to initiate signaling cascades (primarily nuclear factor-κB (NF-κB)-dependent pathway) that ultimately promote a transcriptional response to upregulate pro-inflammatory mediators (Figure 1).

K^+ efflux is recognized as a crucial upstream signal for NLRP3 inflammasome activation (Zhou et al., 2020). Activation of NLRP3 also requires the mobilization of Ca^{2+} (Diaz-Del-Olmo et al., 2021). Mobilization of Ca^{2+} occurs when extracellular Ca^{2+} moves across channels in the plasma membrane and the Ca^{2+} stored in the endoplasmic reticulum is released into the cytoplasm, which can induce NLRP3 inflammasome activation (Watanabe et al., 2020; Diaz-Del-Olmo et al., 2021). In addition, Cl^- was implicated in NLRP3 activation. Cl^- channel blockers and elevated levels of extracellular Cl^- can inhibit, whereas reduced levels of Cl^- can enhance, the activation of NLRP3 (Zhou et al., 2020). Cl^- efflux may be downstream of K^+ efflux and affects ASC polymerization, whereas K^+ efflux promotes NLRP3 oligomerization (Zhou et al., 2024).

In addition to the ion channels mentioned earlier that can activate the NLRP3 inflammasome, organelle dysfunction can also trigger inflammasome activation (Movahedpour et al., 2023). In a study, sterile lysosomal rupture caused by L-leucyl-L-leucine methyl ester is sufficient to trigger NLRP3 inflammasome activation, whereas suppression of phagosomal acidification or cathepsin B blocks NLRP3 activation (Movahedpour et al., 2023). Mitochondrial dysfunction contributes to the activation of the NLRP3 inflammasome (Kodi et al., 2024). When damaged

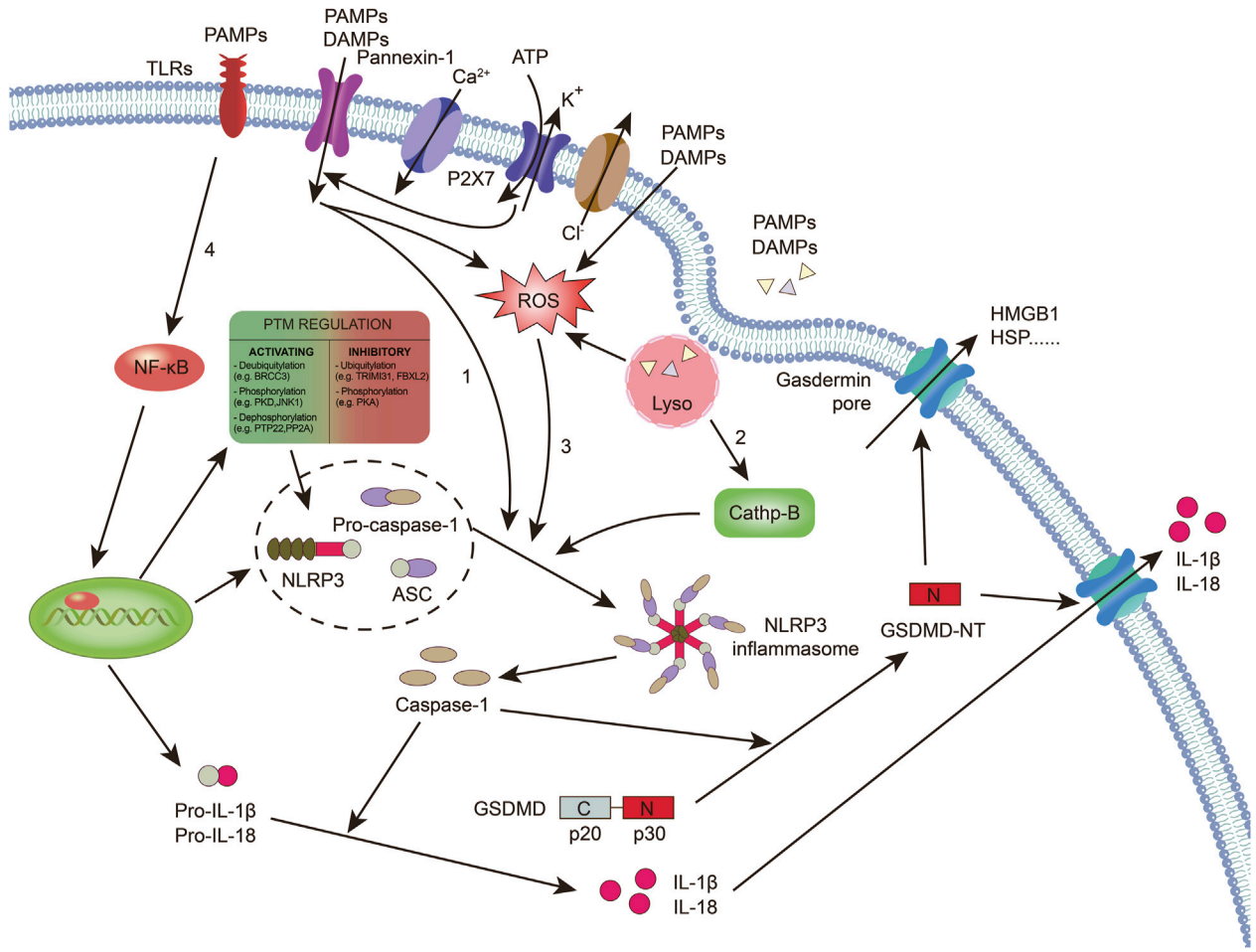

FIGURE 1
Caspase-1-dependent canonical pyroptotic cell death induced by NLRP3 inflammasome activation. The assembly of the NLRP3 inflammasome involves NLRP3 oligomerization and ASC recruitment, triggering the autocleavage of pro-caspase-1. This autocleavage leads to the activation of caspase-1, which converts inactive pro-IL-1β and pro-IL-18 into their bioactive and secreted forms, namely IL-1β and IL-18. Additionally, active caspase-1 cleaves GSDMD, generating GSDMD-NT, which forms pores on the plasma membrane, inducing pyroptosis. Various models have been proposed to explain the assembly of the NLRP3 inflammasome. 1): Extracellular ATP can activate the NLRP3 inflammasome through different mechanisms. This includes the activation of the P2X7 receptor leading to the opening of the pannexin-1 pore, allowing the entry of extracellular factors that directly interact with NLRP3. Alternatively, NLRP3 can sense either the efflux of K+ or the loss of membrane integrity. 2): Crystalline or particulate agonists can be phagocytosed, resulting in the release of lysosomal cathepsins B and L, which are detected by the NLRP3 inflammasome. 3): NLRP3 agonists such as DAMPs and PAMPs can trigger the production of ROS, which activates the NLRP3 inflammasome. It is important to note that these models are not mutually exclusive but rather interact with each other. 4): The activation of the NLRP3 inflammasome also requires "priming" with TLR agonists, such as LPS.. (ASC: apoptosis-associated speck-like protein; TLRs: toll-like receptors; DAMPs: damage-associated molecular patterns; GSDMD: gasdermin D; NT: n-terminal; HSP: heat shock proteins; HMGB-1: high mobility group box-1; IL: interleukin; NLRP3: NOD-like receptor protein 3; NF-κB: nuclear factor-κB; PAMPs: pathogen-associated molecular patterns; P2X7: purinergic receptor P2X, ligand-gated ion channel 7; ROS: reactive oxygen species).

organelles accumulate due to deficiencies in autophagic proteins, dysfunctional mitochondria release mitochondrial DNA, leading to NLRP3 inflammasome activation (Kodi et al., 2024). Additionally, mitochondrial ROS (mtROS) can initiate NLRP3 activation (Kodi et al., 2024). Inhibiting autophagy or mitophagy results in the buildup of mitochondrial ROS and subsequent NLRP3 activation (Chen et al., 2022; Lewisluján et al., 2022; Zhang et al., 2022; Lu et al., 2023).

Post-transcriptional modifications of the NLRP3 protein occur in unstimulated cells and during priming and activation to modulate its activation and function (Swanson et al., 2019). In peritoneal macrophages, tripartite motif 31 ubiquitylates NLRP3, targeting it for proteasomal degradation (Chan and Schroder, 2020). The E3 ubiquitin ligase f-box and leucine-rich repeat protein 2 are suggested to prevent NLRP3 activation by directing it to the proteasome in lung epithelial cells (Han et al., 2020). Additionally, membrane-associated ring-ch-type finger 7, another E3 ubiquitin ligase, inhibits NLRP3 downstream of dopamine-induced D1 receptor signaling (Sandall and Macdonald, 2019). Another regulatory level involves phosphorylation of NLRP3 at Ser291 (or Ser295 in humans) by protein kinase A, leading to K48- and K63-linked ubiquitination and subsequent proteasomal degradation (Ren et al., 2019). Song et al. Demonstrated that following LPS priming, NLRP3 is phosphorylated on Ser194 by c-Jun N-terminal kinase 1, promoting NLRP3 oligomerization upon activation by canonical stimuli (Spalinger et al., 2023). In monocytic

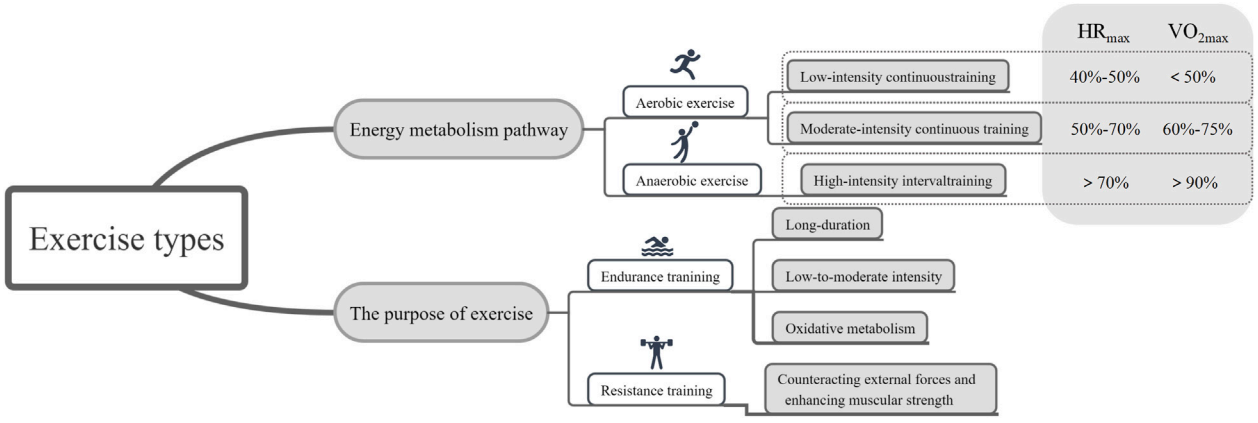

FIGURE 2
Regarding the specific classification of exercise and the criteria for its determination. (HR_{max}: maximum heart rate; VO_{2max}: maximum volume of oxygen).

cells, a study revealed that dephosphorylation of NLRP3 at Tyr861 by protein tyrosine phosphatase nonreceptor 22 induces NLRP3 activation (Spalinger et al., 2020). Whether these pathways synergize to tightly control NLRP3 activity remains elusive.

3 Mechanisms of pyroptosis

Recent advances have shed light on the molecular mechanisms of pyroptosis, a form of programmed cell death induced by the agonist of the NLRP3 inflammasome (McKee and Coll, 2020; Yuan et al., 2020; Chen et al., 2022; Zhong et al., 2023). Among these mechanisms, GSDMD has emerged as a crucial mediator of pyroptosis. GSDMD belongs to a family of proteins named GSDMs, which share a pore-forming domain (McKee and Coll, 2020). Cleavage of GSDMD by caspase-1 or caspase-4/5/11 releases GSDMD-NT, the NT domain of GSDMD (McKee and Coll, 2020). GSDMD-NT then forms these pores in the plasma membrane, leading to cell swelling and osmotic lysis (Ren et al., 2019). Other members of the GSDM family also possess pore-forming activity but are not targeted by inflammatory caspases (Chen et al., 2022). The cleavage of GSDMD occurs at a conserved residue called D276, resulting in the separation of GSDMD into two domains: GSDMD-NT (p30) and GSDMD C-terminal domain (p20) (Zhong et al., 2023). GSDMD-NT can interact with lipids in the plasma membrane and assemble into large oligomeric pores (Yuan et al., 2020). This disruption of the cell membrane integrity leads to an increase in intracellular osmotic pressure and the release of inflammatory intracellular contents, including high mobility group box-1 (HMGB-1) and heat shock protein (Yuan et al., 2020; Chan et al., 2023). This process, characterized by caspase-1-dependent cleavage of GSDMD, is known as the pyroptotic pathway (Chan et al., 2023).

4 Overview of exercise

Exercise refers to the movement of the body, typically involving the musculoskeletal system, to maintain health, improve physical fitness, promote cardiovascular health, and enhance the overall quality of life (Vella et al., 2017). It is a crucial component for maintaining both physical and mental wellbeing (Vella et al., 2017). By selecting forms of exercise that suit individual needs and goals, comprehensive health benefits can be achieved (Vella et al., 2017). The most common classification of exercise is based on the primary energy metabolism pathways, dividing it into aerobic and anaerobic exercise (Paluch et al., 2024) (Figure 2).

Aerobic exercise is a form of activity that generates energy through oxidative metabolism (Mueller et al., 2021). This type of exercise involves relatively low to moderate intensity over an extended period to ensure the body can supply enough oxygen to support energy production (Mueller et al., 2021). Aerobic exercise is characterized by lower intensity, safety, rhythmic, and sustained durations, with relatively minor stress on various organs, reducing the risk of exercise-related injuries (Mueller et al., 2021). Common aerobic exercise programs include low-intensity continuous training (LICT) and moderate-intensity continuous training (MICT) (Troosters et al., 2023). The intensity of exercise is typically measured using parameters such as maximum heart rate and maximum oxygen consumption (Blanks et al., 2019). For maximum heart rate (HR_{max}), low-intensity exercise falls within the 40%–50% range, while moderate-intensity exercise falls within the 50%–70% HR_{max} range (Blanks et al., 2019). Regarding the maximum volume of oxygen (VO_{2max}), values below 60% are considered low intensity, and those between 60% and 75% are considered moderate intensity (Blanks et al., 2019).

Anaerobic exercise involves high-intensity, momentary bursts of muscle activity in an "oxygen-deprived" state (Liao et al., 2022). It is characterized by high-intensity loads and brief durations, making it challenging to sustain for extended periods, and recovery from fatigue is slower (Liao et al., 2022). Anaerobic exercise enhances muscle strength, improves adaptability, and serves as a primary source of muscle growth (Casado et al., 2023). The intensity of anaerobic exercise is relatively high, and the sustainable duration is short, resulting in high-intensity loads that can lead to muscle fatigue and soreness (Casado et al., 2023). Common anaerobic exercise programs include resistance training (RT) and high-

intensity interval training (HIIT) (May et al., 2020). Recently, HIIT has gained popularity as a time-efficient exercise strategy that has been proven to improve cardiovascular risk factors in various populations (May et al., 2020). This training method employs alternating patterns of work and rest to enhance cardiorespiratory endurance, promote fat burning, and provide more efficient training effects in a shorter time (May et al., 2020). High-intensity training is identified by an HR_{max} exceeding 70% or VO_{2max} exceeding 90% (Blanks et al., 2019).

Additionally, based on the primary training goals, exercise can be classified into endurance training (ET) and RT (Consitt et al., 2019). ET involves prolonged, continuous activities at relatively low loads, primarily relying on aerobic metabolism to produce energy (Rothschild and Bishop, 2020). It induces adaptations in the cardiovascular and musculoskeletal systems, supporting overall improvements in exercise capacity and performance (Miko et al., 2020). However, for older individuals who are overweight or obese, especially those with symptoms of osteoarthritis, regular ET may be uncomfortable and painful, necessitating the introduction of alternative forms of exercise (Cutrufello et al., 2020).

RT, on the other hand, offers various health benefits, supports body weight, and avoids imposing impact stress on joints (Schoenfeld et al., 2016). Therefore, RT may be an appealing exercise modality for overweight/obese older individuals. This type of training emphasizes resistance against external forces to enhance muscle strength, endurance, and mass, with each effort specifically targeting the resistance generated, designed to increase muscle strength and explosiveness (Carvalho et al., 2022).

Although most literature suggests that exercise can improve CVD, excessive endurance exercise (EEE) may have many potential adverse effects on cardiac structure and function (O'Keefe et al., 2012). Acutely, EEE can increase myocardial injury markers, lead to chamber dilation, and decrease right ventricular function (Levine, 2014). Chronically, concerns exist that this degree of EEE may lead to detrimental cardiac remodeling and fibrosis, as well as non-lethal arrhythmias, especially an increased risk of atrial fibrillation, and potentially more lethal ventricular arrhythmias (Nath et al., 2023). Recent studies also indicate that despite a more favorable overall profile of coronary heart disease risk in long-distance runners, they may have higher levels of atherosclerosis and coronary heart disease risk (Neumann et al., 2022; O'Keefe et al., 2021). However, the benefits of aerobic exercise on CVD mortality appear to be significantly diminished when running exceeds 30 miles per week or walking exceeds 46 miles per week (Schwartz et al., 2014). Even though that lack of physical activity is more prevalent than EEE in the overall population, the potential adverse effects may be more serious on a societal level for overall health and cardiovascular health (McCullough and Lavie, 2014). These studies also suggest that more is not necessarily better, and even low doses of aerobic exercise, particularly running, seem to confer benefits for long-term cardiovascular health and lifespan.

5 Mechanisms by which exercise regulates NLRP3 inflammasome activation

Exercise has long been acknowledged as an effective intervention in regulating the innate immune response (Pope and Wood, 2020). In general, LICT and MTCT have positive effects on the immune system, whereas HIIT tends to have the opposite effect (Xian et al., 2022). However, there is limited research on how exercise specifically influences the activity of the NLRP3 inflammasome.

From the perspective of material metabolism, many studies have reported that glucose and lipids can directly activate the NLRP3 inflammasome (Vandanmagsar et al., 2011; Zhang et al., 2021; Baik et al., 2023), while exercise can directly alleviate glucose and lipid metabolism, reducing the levels of blood sugar and lipids, thereby improving CVD. There is currently no literature reporting that exercise can directly regulate the activation pathway of NLRP3 inflammasome through glucose and lipid metabolism (Mardare et al., 2016; Ma et al., 2021). Therefore, it boldly speculates that the effect of exercise on NLRP3 inflammasome activation is likely to be exerted through the regulation of glucose and lipid metabolism.

From the mitochondrial standpoint, since most studies suggest that mitochondria are involved in regulating NLRP3 inflammasome activation (Chen et al., 2019; Hong et al., 2021), it is speculated that exercise adaptation of mitochondria may affect the NLRP3 inflammasome activity. Earlier studies demonstrated that MICT significantly decreased the expression of inflammatory cytokines TNF-α, IL-6, and monocyte chemoattractant protein-1 induced by metabolic disorders, coupled with an upregulation in mitochondrial proteins (Liu et al., 2017; Chen et al., 2018). Conversely, HIIT led to mitochondrial dysfunction and an augmentation in the secretion of pro-inflammatory factors (Memme et al., 2021). Moreover, additional investigations indicated that MICT facilitated mitochondrial biogenesis, bolstered antioxidant capacity, and restrained the overactivation of the NLRP3 inflammasome (Mason et al., 2020; Powers et al., 2020). Furthermore, aerobic exercise mitigated cardiac dysfunction by modulating the expression of proteins implicated in mitochondrial quality and NLRP3/caspase-1/IL-1β signaling (Salo et al., 1991). In pathological conditions, mitochondrial damage leads to increased production of mtROS (Chen et al., 2020). The elevated mtROS mediates the activation of the NLRP3 inflammasome through thioredoxin interacting protein (TXNIP) and thioredoxin (Zhang et al., 2021). These findings imply that MICT might diminish mitochondrial ROS production through the regulation of mitochondrial quality control, enhancement of mitochondrial function, and the facilitation of damaged mitochondria clearance. Consequently, this could inhibit the NLRP3 inflammasome pathway and alleviate exaggerated inflammatory responses.

From molecular mechanisms in the cell, the key factors in exercise-mediated improvement of CVD through the NLRP3 inflammasome may be related to the regulation of the NF-κB signaling pathway. Some studies have focused on the NF-κB pathway as a critical node to explore the molecular mechanisms among exercise and NLRP3 inflammasome (Wang et al., 2019; Zhou et al., 2022). For example, research has demonstrated that aerobic exercise is capable of reducing the expression of nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4), ROS, TNF-α, IL-18, NF-κB p65, and the NLRP3 inflammasome (Zhou et al., 2022). These findings suggest that exercise may ameliorate the pathological alterations in diabetes mellitus through the modulation of the NOX4/ROS/NF-κB/NLRP3 inflammasome signaling cascade (Zhou et al., 2022). In a separate study, Wang

et al., 2019 observed that aerobic exercise suppresses the acetylation of forkhead box transcription factor O1 (FOXO1) in the brain tissue of diabetic rats, which in turn promotes the phosphorylation of FOXO1, thus inhibiting expression of NF-κB and NLRP3 inflammasome protein. This downregulation contributes to the inhibition of inflammatory responses, indicating that exercise may exert anti-inflammatory effects via the FOXO1/NF-κB/NLRP3 inflammasome pathway (Wang et al., 2019). Although studies have shown that exercise improves CVD through the NLRP3 inflammasome (Li et al., 2021; Zhou et al., 2022), further research is needed to elucidate the detailed molecular mechanisms by which exercise regulates the NLRP3 inflammasome.

6 Exercise, NLRP3 inflammasome, and CVD

6.1 Exercise improves AS and inhibits NLRP3 inflammasome

The pathogenesis of AS involves multiple processes, including endothelial dysfunction, low-density lipoprotein accumulation and oxidation, monocyte and lymphocyte recruitment, smooth muscle cell migration and proliferation, proinflammatory cytokine activation, and platelet adhesion (Zeng et al., 2019). Notably, the augmented release of inflammatory cytokines primarily attributed to endothelial cells, macrophages, and smooth muscle cell pyroptosis or NLRP3 inflammasome activation significantly contributes to AS formation and development (Zhaolin et al., 2019). Specifically, NLRP3 inflammasome-dependent pyroptosis triggers endothelial dysfunction, thereby acting as a catalyst for AS in these cells (Sun et al., 2017). This highlights the crucial role of NLRP3 inflammasome in promoting the release of inflammatory mediators and contributing to the pathological changes associated with AS.

Exercise can reduce the inflammatory death of local endothelial cells, slowing the development of AS plaques (Xu et al., 2019). A study found that voluntary wheel running, a natural type of aerobic exercise in the murine model could decrease the protein levels of the inflammasome components and markedly inhibit the caspase-1 activity in endothelial cells within the aorta of mice fed with a high-fat diet (HFD) (Lee et al., 2020). Recently, another study has demonstrated that exercise-induced a significant downregulation of m6A modification and methyltransferase-like 14 (Yang et al., 2023). This protein binds to the m6A sites of nuclear paraspeckle assembly transcript 1 (NEAT1) and promotes NEAT1 expression through subsequent YT521-B homology domain-containing 1, which transcriptionally promotes NLRP3 expression and endothelial pyroptosis (Yang et al., 2023). As a result, exercise effectively inhibits NLRP3 expression and endothelial pyroptosis, preventing AS plaque formation (Yang et al., 2023). In addition, it is widely recognized that fibroblast growth factor 21 (FGF21), a well-established negative risk factor for AS expressed in the aorta, exerts inhibitory effects on NLRP3 inflammasome activity, thereby attenuating aortic pyroptosis to prevent AS development (Zeng et al., 2020). Interestingly, aerobic exercise increases FGF21 sensitivity while downregulating the expression of pyroptosis-related proteins mediated by NLRP3 inflammasome in the aorta (Li et al., 2022). These findings suggest that activated FGF21 may be involved in aerobic exercise inhibiting NLRP3 inflammasome-mediated pyroptosis in the aorta. However, the current study has not investigated whether exercise improved AS via inhibiting NLRP3 inflammasome and pyroptosis in these cells, which needs further research in the future.

6.2 Exercise improves HHcy and inhibits NLRP3 inflammasome

Homocysteine (Hcy), a non-essential amino acid sulfur, is derived from methionine and is used for methylation of DNA/RNA methylation (Veeranki and Tyagi, 2013). HHcy refers to a condition where plasma Hcy levels exceed 15 μmol/L and has been associated with various diseases especially AS (Winchester et al., 2014). HHcy promotes the generation of ROS through mechanisms such as mixed disulfide formation and auto-oxidation (Veeranki and Tyagi, 2013; Winchester et al., 2014). Recent research has demonstrated that HHcy-induced activation of the NLRP3 inflammasome in macrophages contributes to vascular inflammation and AS by activating caspase-1-mediated pyroptosis (Wang et al., 2017). In addition, acid sphingomyelinase upregulation by Hcy promotes clustering of lipid rafts mediating the assembly of NADPH oxidase complex resulting in increased ROS generation followed by NLRP3 inflammasome activation leading to pyroptosis contributing towards the development of AS (Liu et al., 2022).

Although direct evidence is currently lacking regarding whether exercise specifically modulates NLRP3 inflammasome activation or pyroptosis about HHcy, it has been observed that exercise can lower Hcy levels (Liu et al., 2022). In a folate-restricted model of HHcy, exercise can reduce the increase in plasma Hcy levels by increasing betaine Hcy s-methyltransferase levels in the kidneys and promoting nonclassical remethylation to convert Hcy into methionine (Vincent et al., 2006; Neuman et al., 2013). Moreover, apart from reducing plasma Hcy levels, exercise holds the potential for mitigating Hcy-induced lipid peroxidation and ameliorating reductions in superoxide dismutase and catalase activity, both of which are implicated in the progression of AS (Neuman et al., 2013). These works suggest that exercise might also inhibit NLRP3 inflammasome activation or pyroptosis as a part of its overall impact on reducing HHcy-related complications. Given these findings, further investigation is warranted to explore whether exercise precisely influences NLRP3 inflammasome activation or pyroptosis and their role in HHcy management. Understanding this relationship could provide valuable insights into developing targeted interventions for individuals with elevated Hcy levels and associated CVD.

6.3 Exercise improves obesity and inhibits NLRP3 inflammasome

Obesity, commonly associated with chronic low-grade inflammation, is characterized by the pathological enlargement of adipose tissue (AT) primarily due to excess energy accumulation as fat (Spalding et al., 2008; Wada et al., 2017). Mice with HFD-induced

obesity exhibit increased expression of caspase-1, ASC, and NLRP3. However, knocking out the *Nlrp3* or *Caspase-1* gene suppresses obesity-induced fat depot (Stienstra et al., 2011). Therefore, targeting the activation of NLRP3 inflammasome or pyroptosis represents a promising approach for improving obesity.

A study found that exercise decreased protein expression of inflammasome components (NLRP3 and caspase-1) in bone marrow-derived macrophages (BMDM) and AT isolated from mice with diet-induced obesity (Javaid et al., 2021). Another study investigated perform mice fed either a standard diet or an HFD and subjected to regular ET or RT and discovered that RT attenuated increased NLRP3 expression and reduced levels of IL-18 in isolated AT, while ET effectively reduced the expression of TNF-α and IL-18 in supernatant from AT, suggesting that exercise can reduce inflammasome activation in ATs and achieve systemic downregulation of inflammatory cytokines (Wada et al., 2017). Besides AT, endothelial dysfunction emerges early on in CVD associated with obesity (Stienstra et al., 2011; Wada et al., 2017). A study demonstrated that engaging in voluntary running while on an HFD led to a significant reduction in active caspase-1 levels within the endothelial cells lining the aorta when compared to sedentary mice on the same diet (Li et al., 2023). These findings indicate that voluntary running alleviates impaired blood vessel function via inhibiting NLRP3 inflammasome activation (Li et al., 2023). In addition to endothelial cells, exercise suppressed NLRP3 inflammasome activation as revealed by downregulated IL-1β and IL-18 in BMDM (Li et al., 2023). This body of research demonstrates that exercise exerts inhibitory effects on NLRP3 inflammasome activation across various cell types to ameliorate obesity.

It's worth noting that a human study found that exercise reduces plasma IL-18 levels in obese individuals, indirectly suggesting the inhibition of the NLRP3 pathway through exercise to improve obesity (Stienstra et al., 2011). Furthermore, 8-week high-intensity and aerobic interval training (three times/week) in men and women with metabolic syndrome resulted in decreased IL-18 mRNA levels in abdominal AT and a numerical decrease in plasma IL-18 concentration (Stienstra et al., 2011; Stienstra et al., 2011; Stienstra et al., 2011; Stienstra et al., 2011). Similarly, a pilot study, conducted among thirty-seven obese individuals demonstrates that exercise intervention, primarily consisting of activities such as walking, jogging, and functional exercise circuits designed to enhance aerobic capacity and speed, leads to significant reductions in ASC mRNA expression levels when compared to the hypocaloric group without any form of exercise participation (Barrón et al., 2020). These findings indicate an inverse relationship between ASC mRNA expression and aerobic interventions. Another randomized controlled trial involving 36 obese inactive subjects further delineated the type of exercise intervention and demonstrated both HIIT and MICT significantly reduced NLRP3 gene expression in serum samples from all subjects, strongly suggesting that diversity intensity interval training can inhibit NLRP inflammasome in obese (Armannia et al., 2022).

Therefore, exercise is considered a crucial strategy for reducing inflammation and metabolic disorders associated with obesity. Further research will enhance understanding of the complex relationship between exercise and NLRP3 inflammasome, leading to more effective interventions for managing obesity-related diseases.

6.4 Exercise improves diabetes and inhibits NLRP3 inflammasome

Diabetes is a metabolic disease that poses a significant threat to human health. Type 2 diabetes, a significant risk factor for both microvascular and macrovascular diseases, accounts for 90% of diabetes cases and is a leading cause of death, particularly due to coronary heart disease (Einarson et al., 2018). Inflammatory processes play a crucial role in the development of complications associated with diabetes (Roncero-Ramos et al., 2018). Recently, NLRP3 inflammasome and pyroptosis have emerged as key contributors to insulin resistance (IR) (Roncero-Ramos et al., 2018). Peripheral blood-derived macrophages from drug-naïve patients with type 2 diabetes show increased expression of NLRP3 and ASC along with caspase-1 activation and IL-1β maturation (Lee et al., 2013). *In vivo* and *in vitro* studies have reported that high glucose stimulates IL-1β secretion in pancreatic β cells, resulting in their death through the activation of NLRP3 inflammasome (Zhou et al., 2010; Wu et al., 2019). The various modes of activating the NLRP3 inflammasome are fundamental factors influencing its complex effects on the progression of type 2 diabetes (Chen et al., 2021).

Engaging in aerobic exercise can improve IR and reduce the expression of NLRP3 and IL-1β in individuals with type 2 diabetes in aortic tissue, suggesting its positive impact on IR by inhibiting NLRP3 inflammasome (Hassanpour Soleimani et al., 2021). HMGB1 is a major pro-inflammatory cytokine released as a result of pyroptosis (Biguetti et al., 2019). This work also found that exercise can reduce the secretion of HMGB1, which is significantly increased in individuals with diabetes and contributes to disease progression (Hassanpour Soleimani et al., 2021). Previous studies have shown that exercise has the potential to lower HMGB-1 levels in circulation and tissues of diabetic patients, possibly by inhibiting NLRP3 inflammasome activation and pyroptosis (Haß et al., 2022; Haß et al., 2023). Furthermore, exercise decreased circulating levels of IL-1, which may potentially protect against IL-1-mediated destruction of β-cells (Stumvoll et al., 2005). Although there is currently no direct evidence on whether aerobic exercise specifically affects NLRP3 inflammasome activation or pyroptosis and thus decreases levels of blood glucose, these findings highlight the significant role played by exercise in regulating IR through its impact on NLRP3 inflammasome and pyroptosis.

6.5 Exercise improves DCM and inhibits NLRP3 inflammasome

DCM is a distinct cardiac phenotype observed in diabetic patients characterized by structural changes such as cardiac hypertrophy, cardiomyocyte death, and fibrosis, as well as functional abnormalities (Sun et al., 2021). The molecular mechanisms underlying DCM involve various factors including hyperglycemia, IR, fatty acids, oxidative stress, mitochondrial dysfunction, inflammation, and endothelial dysfunction (Chen et al., 2020). In particular, inflammation is believed to be present in the early stages of diabetes and plays a crucial role in promoting DCM (Luo et al., 2017). Emerging evidence has verified that

NLRP3 inflammasome-mediated cardiomyocyte pyroptosis is a key participant in DCM (Xie et al., 2020). Human diabetic hearts also exhibit elevated activation of the NLRP3 inflammasome and cardiac pyroptosis compared to non-diabetic heart tissues (Zhang et al., 2015; Xie et al., 2020). Moreover, high glucose levels (35 mM) significantly induce increased protein expression of NLRP3, caspase-1, and IL-1β accompanied by noticeable cardiomyocyte pyroptosis, leading to loss of contractile units and cardiac dysfunction (Zhang et al., 2015). In contrast, silencing the *Nlrp3* gene ameliorates cardiac inflammation and pyroptosis and improves cardiac function by ameliorating cardiac inflammation and pyroptosis both *in vivo* and *in vitro* experiments (Chen et al., 2020).

Exercise intervention has been shown to effectively prevent and treat DCM by modulating the NLRP3 inflammasome. For example, the expressions of NLRP3, caspase-1-p20, caspase-1p20/caspase-1, and IL-1β were increased in the myocardium of HFD-induced obese mice (Lee et al., 2018). However, treadmill exercise inhibited these parameters (Sun et al., 2021). This demonstrates that exercise training can prevent obesity-induced cardiac inflammasome formation, pyroptosis activation, and pro-inflammatory response (Sun et al., 2021). In addition, recent studies in DCM mice have demonstrated that although exercise has a limited impact on interstitial fibrosis, it can effectively reverse cardiac dysfunction by reducing the activity of NLRP3 inflammasome and inhibiting pyroptosis (Takahashi, 2019; Zhang et al., 2019). Moreover, in DCM rat models, elevated expression levels of the P2X7 receptor, NLRP3, caspase-1, and serum IL-1β were observed in the myocardium (Wang et al., 2022). However, following a 12-week treadmill running regimen in these rats, improvements were observed in terms of collagen deposition, cell disorder, as well as the expression levels of NLRP3, caspase-1, P2X7 receptor, and IL-1β within their heart (Zhang et al., 2015). Similarly, aerobic exercise also can inhibit the thioredoxin interacting protein (TXNIP)/NLRP3 inflammasome pathway and alleviate endothelial dysfunction in atherosclerotic coronary arterioles (Hong et al., 2018). These findings suggest that aerobic exercise can effectively mitigate fibrosis in the hearts subjected to an HFD and inhibit the activation of the NLRP3 inflammasome and pyroptosis in the myocardium. The effectiveness of exercise intervention on the NLRP3 inflammasome depends on the duration and intensity of exercise (Hong et al., 2018; Khakroo et al., 2019). Chronic exercise with moderate intensity significantly decreases the expression of the NLRP3 gene and levels of IL-1β, and IL-18 cytokines in serum (Khakroo et al., 2019). Conversely, chronic exercise with high intensity leads to a significant increase in gene expression of NLRP3 and levels of IL-1β, and IL-18 cytokines in serum (Khakroo et al., 2019). Therefore, personalized exercise regimens are necessary as there are currently no available guidelines; further research is needed.

6.6 Exercise improves hypertension and inhibits NLRP3 inflammasome

Hypertension is a potentially fatal yet preventable risk factor for CVD and accounts for the majority of cardiovascular mortality (Deussen and Kopaliani, 2023). Persistent inflammation plays a pivotal role in hypertension development, with activation of the inflammasome and pyroptosis being potential contributors to its onset (De et al., 2021). NLRP3 inflammasome activities have been implicated in various cell types associated with pulmonary hypertension, including pulmonary arterial smooth muscle cells, pulmonary artery endothelial cells, and systemic hypertension (Haß et al., 2023). Additionally, higher serum levels of IL-1β were observed in patients with hypertension compared to normotensive controls (Wu et al., 2022).

Exercise is commonly suggested as a lifestyle adjustment for individuals with hypertension due to various factors including inhibition of inflammation (Newman and Verdin, 2014; Chakraborty et al., 2018; Kong et al., 2021). β-Hydroxybutyrate (β-OHB) ester is primarily synthesized in the liver and transported to extrahepatic tissues, traditionally recognized as a crucial metabolic fuel during starvation periods (Newman and Verdin, 2014). Contemporary evidence indicates that ketone bodies like β-OHB can maintain physiological homeostasis by inhibiting NLRP3-inflammasome-mediated inflammation (Kong et al., 2021). Recently, a non-targeted metabolomics approach revealed nutritional intervention with β-OHB reversed the high salt-induced adverse effects including renal NLRP3-mediated inflammation, fibrosis, and hypertension (Chakraborty et al., 2018). Interestingly, exercise is associated with increased circulating levels of β-OHB (Luo et al., 2021). Therefore, exercise may raise β-OHB levels and subsequently inhibit renal NLRP3 inflammasome activation, thereby alleviating hypertension and preserving kidney function. Another *in vivo* study provided direct evidence of the impact of exercise training on the downregulation of NF-κB and NLRP3 pathways in the mesenteric artery of spontaneously hypertensive rats (SHR) (Luo et al., 2021), which revealed that three intensity training intensities from low to high significantly inhibited the expression of NLRP3 inflammasome component and NF-κB within the mesenteric artery and alleviate BP in SHR rat (Luo et al., 2021). In addition to the SHR rat model, Bal et al., 2022 discovered that regulated aerobic exercise also effectively reduces BP and suppresses protein expression of NLRP3, IL-1β, and caspase-1 in the heart within the deoxycorticosterone-acetate salt hypertension model. These findings from the diversity hypertension model demonstrate that exercise training effectively attenuates NLRP3 inflammasome activity, highlighting its potential as a therapeutic intervention for hypertension.

6.7 Exercise improves MI and inhibits NLRP3 inflammasome

MI, a common condition, refers to the death of heart muscle cells caused by a blockage in the coronary arteries (Wang et al., 2020). Following a heart attack, inflammatory reactions occur, characterized by the release of inflammatory cells, cytokines, and chemical hormones (Mezzaroma et al., 2011; Wang et al., 2020). These processes contribute to cardiac dysfunction, damage to the heart muscle, and remodeling (Mezzaroma et al., 2011; Wang et al., 2020). Indeed, NLRP3 inflammasome and pyroptosis-mediated inflammation have been reported to contribute to MI progression (Sandanger et al., 2013). Sandanger et al., 2013 first showed an

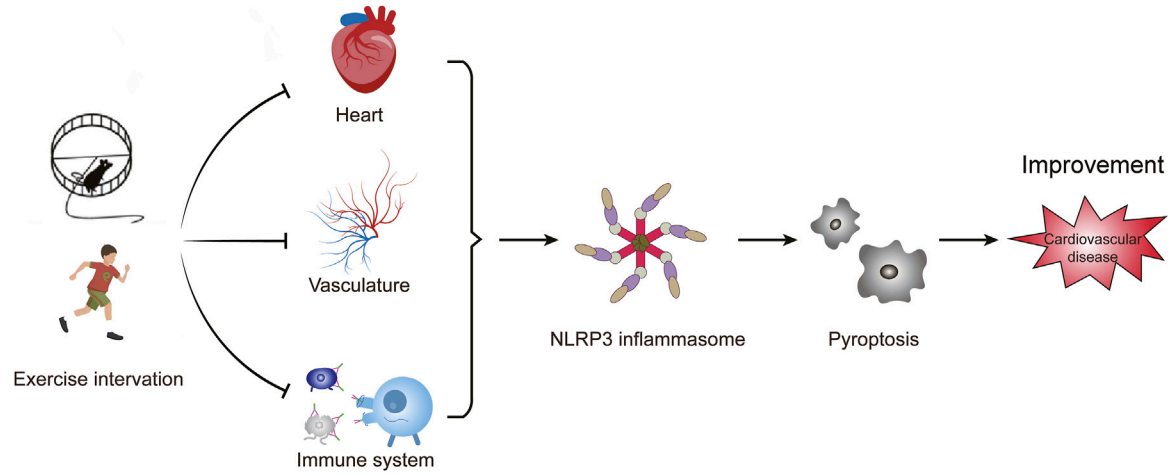

FIGURE 3
Exercise intervention affects cardiovascular disease by NLRP3 inflammasome-mediated pyroptosis. The activation of NLRP3 inflammasome induces pyroptosis, which promotes vascular inflammation and heart remodeling, and ultimately leads to cardiovascular diseases. Exercise affects the progression of cardiovascular diseases by inhibiting the activation of the NLRP3 inflammasome.

increase in NLRP3 inflammasome activity in the left ventricle of the heart after MI. Furthermore, it appears that interfering with NLRP3 inflammasome signaling can prevent and mitigate damage caused by MI (Sandanger et al., 2013).

The research has shown that exercise training can delay the onset of ischemic reperfusion injury and MI (Thomas et al., 2016). In other words, exercise acts like ischemia preconditioning, which stimulates beneficial cellular responses. Azam Ahmadi et al., 2022 found HIIT can effectively decrease heart injury and NLRP3 expression in rats with MI. Interestingly, dynamin-related protein 1 (Drp1), an essential mitochondrial fission protein, can activate NLRP3 inflammasome through ROS generation after MI (Ahmadi et al., 2022; Jiang et al., 2022). However, the Azam Ahmadi team's work demonstrates exercise preconditioning (EP) in a short period did not affect Drp1 (Ahmadi et al., 2022), while inhibiting NLRP3 expression. This finding indicates that EP in a short period is required to inhibit NLRP3 inflammasome independently of Drp1; the reduction of NLRP3 can occur through other mechanisms (Jiang et al., 2022).

6.8 Exercise affects HF and regulates NLRP3 inflammasome

HF means that the heart is unable to pump sufficiently to sustain blood flow to meet the needs of the body (Sacco et al., 2014). It represents the advanced stage of various CVD, including myocarditis, MI, cardiomyopathy, hypertension, atrial fibrillation, valvular heart disease, alcohol abuse, and infection (Bracey et al., 2013). Recent studies on calcineurin transgene mice have shown elevated mRNA of NLRP3 and enhanced cleavage of caspase-1 along with cardiac hypertrophy and ventricular dilatation (Bracey et al., 2013; Napodano et al., 2023). Genetic ablation of NLRP3 or administration of IL-1 receptor antagonist has been found to attenuate cardiac inflammation and systolic dysfunction (Bracey et al., 2013; Zhang et al., 2014; Napodano et al., 2023). Additionally, activation of NLRP3 inflammasome has been observed in LPS-stimulated cardiac fibroblasts and myofibroblasts, suggesting that its potential contribution to myocardial dysfunction through NLRP3 inflammasome or pyroptosis (Zhang et al., 2014; Boza et al., 2016). Although more research is needed to fully understand how the NLRP3 inflammasome or pyroptosis contributes to HF pathogenesis and progression; recent studies indicate that targeting this pathway could lead to new treatments for this debilitating condition (Lu et al., 2022; Xin et al., 2022).

Studies have shown that EP can regulate the NLRP3 inflammasome, reducing downstream inflammatory cytokines and protecting the heart (Li et al., 2020; Ma et al., 2021; Zhang et al., 2022). Li et al., 2020 found that moderate-intensity EP is more effective in protecting cardiac function and inhibiting the expression of TXNIP, NLRP3, NF-κB p65, and caspase-1 in the heart. As expected with moderate-intensity exercise, 8 weeks of aerobic exercise at this level inhibited protein expression related to myocardial NLRP3/caspase-1/IL-1β signaling pathways in mice with myocardial hypertrophy (Ma et al., 2021). In addition, Sandanger et al., 2013 show that the NLRP3 inflammasome is upregulated in myocardial fibroblasts post-MI, and maybe a significant contributor to infarct size development during ischemia-reperfusion. These findings highlight the potential therapeutic value of moderate-intensity exercise for cardiovascular health. However, exhaustive exercise (EE), characterized by sustained high-intensity exercise commonly practiced by athletes and soldiers alike, may lead to adverse reactions such as myocardial inflammation. It is reported that EE can increase NLRP3 expression and induce cardiac dysfunction which can be mitigated by EP at different intensities (Zhang et al., 2015). Importantly, moderate-intensity EP has cardioprotective effects on ventricular systolic and diastolic functions, indicating its superior efficacy compared to other intensities (Zhang et al., 2014).

Importantly, a clinical trial with 54 HF patients showed that exercise increased ASC methylation, decreased IL-1β and ASC

TABLE 1 Types of cells and tissue injury subjected to NLRP3 inflammasome by exercise intervention in various CVD.

Diseases	Exercise type	Specific exercise intervention	Cell types/ Organ	Injury types	Effects	References
AS	Aerobic exercise	Long-distance walking for at least 60 min at least 5 days a week	Human plasma*		NLRP3 inflammasome inactivation	Yang et al. (2023)
AS	Aerobic exercise	Run on a motorized rodent treadmill 5 days a week	Mouse plasma, heart, and aortic endothelial cells	HFD	NLRP3 inflammasome inactivation	(Yang et al., 2023)
AS	Aerobic exercise	Treadmill exercise training for 12 weeks	Serum, the aorta, and the right lobe of the liver	HFD	FGF21 and NLRP3 inflammasome-mediated pyroptosis	Li et al. (2022)
HHcy	Aerobic exercise	Wheel-exercised mice had free access to their wheels 24 h/d for 5 days/wk	Plasma, liver tissue, and kidney	AIN-93G semi-purified diet	Exercise reduced circulating Hcy, which may affect NLRP3 inflammasome-mediated pyroptosis	Neuman et al. (2013)
Obesity	Endurance training or resistance training	at 0.15 m/s, increasing every 3 min by 0.05 m/s; for 5 times/week for 3 min and 3 series	Mouse adipose tissue and serum	HFD	NLRP3 expression, levels of TNF-α and IL-18	Mardare et al. (2016)
Obesity	Aerobic exercise	Treadmill exercise for 8 weeks	Adipose tissue and bone marrow-derived macrophages	HFD	NLRP3 inflammasome	Javaid et al. (2021)
Obesity	Aerobic exercise	Running wheel access	Endothelial cells of the aorta	HFD	Activation of NLRP3 inflammasome	Lee et al. (2013)
Obesity	Aerobic exercise and resistance training	High-intensity aerobic interval training and strength training	Human serum*		IL-18, IL-6, and TNF-α	Stensvold et al. (2012)
Obesity	Aerobic exercise	12 weeks of intervention with exercise	Human serum*	4 subjects with metabolic syndrome	IL-18	(Troseid et al., 2009)
Obesity	Aerobic exercise	Hypocaloric diet exercise	Human peripheral blood*		ASC gene expression and IL-1β	Barrón et al. (2020)
Obesity	Aerobic exercise	High-intensity interval and moderate-intensity continuous training	Human serum samples*		The expression of NLRP3	Armannia et al. (2022)
Diabetes	Aerobic exercise	A treadmill for 6 weeks, 5 sessions per week	Rats adipose tissue and aortic tissue sampling	Streptozotocin	HMGB1 gene expression	Hassanpour et al. (2021)
DCM	Aerobic exercise	A motor treadmill running speed was increased until the speed reached 10 m/min	The heart and serum	HFD	The expression of P2X7R, NLRP3, caspase-1 and IL-1β	Chen et al. (2021)
DCM	Aerobic exercise	1 h of running on a motor-driven treadmill at 15 m/min at a 5° grade, 5 days/week for 15–16 weeks	Heart tissue and isolated coronary arteriole	A western atherogenic diet (0.2% cholesterol, 42% Kcal from fat)	NLRP3 inflammasome inactivation	Hong et al. (2018)
Hypertension	Aerobic exercise	High-intensity exercise	Serum and kidneys	High salt-diet	Upregulated β-OHB	Chakraborty et al. (2018)
Hypertension	Aerobic exercise	A treadmill for 14 weeks, 5 times a week and 60 min each time	Blood, vascular tissue samples, and mesenteric artery	Wistar Kyoto rats	NF-κB/NLRP3 inflammatory pathway	Luo et al. (2021)
Hypertension	Aerobic exercise	A 45-min running on a horizontal treadmill at 20 m/min, 5 days per week	Systolic blood pressure and the left ventricle tissues	Deoxycorticosterone-acetate and salt administration	NLRP3 inflammasome activation	Bal et al. (2022)

(Continued on following page)

TABLE 1 (*Continued*) Types of cells and tissue injury subjected to NLRP3 inflammasome by exercise intervention in various CVD.

Diseases	Exercise type	Specific exercise intervention	Cell types/ Organ	Injury types	Effects	References
MI	Aerobic exercise and anaerobic exercise	10*1-min running intervals were separated by a 2-min rest	Blood samples from the heart and heart tissue	Isoproterenol	NLRP3 inflammasome	Ahmadi et al. (2022)
HF	Aerobic exercise	Low-, middle-, and high-intensity exercise	Serum and myocardial specimens	refer to Bedford's motion load standard	NF-κB p65/ NLRP3 inflammatory	Li et al. (2020)
HF	Aerobic exercise	2, 4, and 8 weeks of moderate-intensity aerobic exercise	Mouse heart tissues	Transverse aortic constriction	NLRP3/caspase-1/IL-1β signaling pathways	Ma et al. (2021)
HF	Aerobic exercise	A treadmill for 90 min/ day and 6 days/week for 6 weeks at a velocity of 15 cm/s	Mouse hearts and primary neonatal mouse cardiomyocytes	Isoprenaline	NLRP3 inflammasome	Zhang et al. (2022)
HF	Aerobic exercise	A progressive, moderate intensity aerobic protocol	Human plasma*		IL-1β and ASC mRNA gene expression	Butts et al. (2018)
HF	Aerobic exercise	Three times a week, 60 min/session, for 8 months	Human serum*		Levels of TNF-α, IL-1β, IL-8, IF-γ and IL-10	Rodrigues et al. (2020)

*Indicated this study with cells or tissue from humans.

ASC, apoptosis-associated speck-like protein; AS, atherosclerosis; DCM, diabetic cardiomyopathy; FGF21, fibroblast growth factor 21; HFD, high-fat diet; HMGB-1, high mobility group box-1; Hcy, homocysteine; HHcy, hyperhomocysteinemia; HF, heart failure; IL, interleukin; β-OHB, β-Hydroxybutyrate; MI, myocardial infarction; NLRP3, NOD-like receptor protein 3; NF-κB, nuclear factor-κB; P2X7, purinergic receptor P2X, ligand-gated ion channel 7; AIN-93G, american institute of nutrition-93 growth).

mRNA levels compared to the control group in plasma, suggesting that exercise may improve HF via epigenetic regulation of ASC (Butts et al., 2018). Recently, our research team has reported for the first time that NLRP3 inflammasome-mediated pyroptosis contributes to the pathogenesis of non-ischemic dilated cardiomyopathy in cellular, murine, and human models (Zeng et al., 2020). Furthermore, numerous studies have investigated exercise intervention in patients with dilated cardiomyopathy and have demonstrated its safety and efficacy in improving exercise capacity and quality of life among these individuals (Stolen et al., 2003; Beer et al., 2008). Consequently, it is meaningful to investigate whether exercise can exert inhibitory effects on NLRP3 inflammasome activation and subsequent pyroptotic cell death in dilated cardiomyopathy. Preliminary data suggest that exercise may effectively inhibit NLRP3 inflammasome activation in dilated cardiomyopathy (unpublished data).

Although these experiments indicate that exercise improves HF by inhibiting NLRP3 inflammasome activation, there is still debate (Butts et al., 2018). For example, previous work reported that exercise training consisting of three sessions per week lasting for 60 min each, including aerobic exercises, strength exercises targeting major muscle groups, and stretching exercises have no effect on serum levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-8, and monocyte chemoattractant protein-1) of patients with severe Chagas cardiomyopathy (Rodrigues Junior et al., 2020), suggesting that exercise training may benefit patients with severe Chagas cardiomyopathy independent of its impact on inflammasome (Rodrigues Junior et al., 2020).

Most studies suggest that exercise improves HF by inhibiting the activation of NLRP3 inflammasomes. Nevertheless, a minority of research suggests that engaging in activities like EE might potentially worsen the progression of HF. It is imperative for future investigations to thoroughly examine the role played by NLRP3 inflammasome activation in exercise-induced exacerbation of HF.

7 Summary and prospects

Exercise plays an important regulatory role in the development of various CVD by influencing the activation of the NLRP3 inflammasome (Figure 3). This impact has been observed in different types of cells and tissues, both *in vivo* and *in vitro* models of CVD (Table 1). This review highlights the close relationship between aerobic exercise, NLRP3 inflammasome, and CVD. It suggests that aerobic exercise can alleviate pyroptosis and improve cardiovascular-related diseases by modulating the NLRP3 inflammatory signaling pathway. Different patterns of exercise have varying impacts on the NLRP3 inflammasome; therefore, further research is needed to determine their optimal effects on specific types of cells and their underlying molecular mechanisms involving the NLRP3 inflammasome. Currently, there is a greater emphasis on animal experiments investigating the influence of exercise on NLRP3 inflammasome or cell pyroptosis while human studies are limited with small sample sizes in this area. Conducting comprehensive research on humans would provide a scientifically sound basis for understanding how exercise regulates NLRP3 inflammasome activity and promotes overall health. Hence, there is a need for methodologically rigorous large-scale human studies to determine ideal patterns of exercise.

The investigation of drugs targeting the NLRP3 inflammasome in clinical trials for CVD has been conducted. For example, the canakinumab anti-inflammatory thrombosis outcome study trial demonstrated the efficacy of IL-1-targeting therapy in preventing

atherothrombotic events, indicating the potential of targeting the NLRP3 inflammasome (Ma et al., 2021; Ridker and Rane, 2021). However, the high cost of canakinumab limits its widespread use in the future. Additionally, long-term suppression of IL-1 signaling may have adverse effects such as increased susceptibility to infections and disturbances in immune homeostasis (Hettwer et al., 2022). Common cardiovascular drugs such as statins, beta-blockers, sodium-glucose cotransporter 2 inhibitors, and glucagon-like peptide 1 agonists may modulate NLRP3 inflammasome activity through different mechanisms, exerting protective effects in CVD (Terentes-Printzios et al., 2022). However, these classical medications carry certain side effects on gastrointestinal function and are prone to causing symptoms like hypotension and arrhythmias (Vaiciuleviciute et al., 2021). Interestingly, research suggests that exercise effectively modulates NLRP3 inflammasome activation, leading to improvements in AS and other CVD (Vaiciuleviciute et al., 2021; Terentes-Printzios et al., 2022). This therapeutic approach is relatively simple, feasible, and considered safe compared to using NLRP3 inhibitors and classical medications for CVD. Therefore, combining medication treatment with exercise intervention offers a potential strategy to improve the quality of life for patients.

Author contributions

PD: Data curation, Writing–original draft, Investigation. YS: Data curation, Writing–original draft, Investigation. YY: Investigation, Resources, Supervision, Writing–review and editing. CZ: Conceptualization, Funding acquisition, Investigation, Supervision, Visualization, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

ASC	apoptosis-associated speck-like protein
AS	atherosclerosis
AT	adipose tissue
BMDM	bone marrow-derived macrophages
BP	blood pressure
CVD	cardiovascular diseases
CARD	caspase recruitment domain
DAMPs	damage-associated molecular patterns
DCM	diabetic cardiomyopathy
Drp1	dynamin-related protein 1
ET	endurance training
EP	exercise preconditioning
EE	exhaustive exercise
EEE	excessive endurance exercise
FGF21	fibroblast growth factor 21
FOXO1	forkhead box transcription factor O1
GSDMD	gasdermin D
NT	n-terminal
NOX4	nicotinamide adenine dinucleotide phosphate oxidase 4
HFD	high-fat diet
HMGB-1	high mobility group box-1
Hcy	homocysteine
HHcy	hyperhomocysteinemia
HIIT	high-intensity interval training
HF	heart failure
IL	interleukin
LRR	leucine-rich repeat
LICT	low-intensity continuous training
IR	insulin resistance
LPS	lipopolysaccharide
β-OHB	β-Hydroxybutyrate
MICT	moderate-intensity continuous training
MI	myocardial infarction
mtROS	mitochondrial ROS
HR_{max}	maximum heart rate
VO_{2max}	maximum volume of oxygen
NLRP3	NOD-like receptor protein 3
NLR	nucleotide-binding domain-like receptor
NF-κB	nuclear factor-κB
NEAT1	nuclear paraspeckle assembly transcript 1
PAMPs	pathogen-associated molecular patterns
PYD	pyrin domain
P2X7	purinergic receptor P2X, ligand-gated ion channel 7
ROS	reactive oxygen species
RT	resistance training
SHR	spontaneously hypertensive rats
TGN	trans-golgi network
TXNIP	thioredoxin interacting protein

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EDITED BY
Yusof Kamisah,
Universiti Kebangaan Malaysia, Malaysia

REVIEWED BY
Baskaran Stephen Inbaraj,
Fu Jen Catholic University, Taiwan
Prabuddha Bhattacharya,
West Bengal State University, India

*CORRESPONDENCE
Lei Zhu,
zhuleimail@zmu.edu.cn
zhuleilei@live.cn

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Amentoflavone for treating cardiocerebrovascular diseases and neurological disorders

Hang Zhang[1,2,3], Yin-mei Ban[1,2,3], De-mei Li[1,2,3], Gang Wang[1,2,3], Juan Gu[1,4] and Lei Zhu[1,2,3]*

[1]Department of Clinical Pharmacy, Key Laboratory of Basic Pharmacology of Guizhou Province and School of Pharmacy, Zunyi Medical University, Zunyi, Guizhou, China, [2]Key Laboratory of Basic Pharmacology of Ministry of Education and Joint International Research Laboratory of Ethnomedicine of Ministry of Education, Zunyi Medical University, Zunyi, Guizhou, China, [3]The Key Laboratory of Clinical Pharmacy of Zunyi City, Zunyi Medical University, Zunyi, Guizhou, China, [4]Department of Pharmacy, Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou, China

Amentoflavone (AME) is a flavonoid compound found in over 120 plants. Its extensive pharmacological activity for treating cardiocerebrovascular diseases and neurological disorders have attracted the attention of researchers in recent years. However, owing to the poor solubility and low bioavailability of AME, it has not been developed as a drug for treating these diseases. This review focuses on two aspects of AME: First, it provides a detailed summary and introduction to AME based on its chemical structure, physicochemical properties, plant sources, extraction and purification methods, administration systems, and pharmacokinetic properties. Second, it summarizes the effects of AME on cardiocerebrovascular diseases and neurological disorders, and its specific pharmacological mechanisms. This review aims to promote the use of AME for treating cardiocerebrovascular diseases and neurological disorders. AME exhibits multiple activities, indicating its potential as a natural drug for treating these diseases. Further studies on its pharmacokinetics and toxicology are required to ensure its safety and efficacy.

KEYWORDS

amentoflavone, cardiovascular diseases, cerebrovascular diseases, neurological diseases, pharmacological effects, anti-inflammatory agents, Chinese herbal medicine

1 Introduction

Cardiocerebrovascular diseases refer to a group of conditions that affect the heart, brain, and other tissues. This category includes both cardiovascular and cerebrovascular diseases, as well as a variety of ischemic and hemorrhagic conditions. These diseases are precipitated by factors such as hyperlipidemia, thickened blood, atherosclerosis, and hypertension (Yan and Guo, 2022). Cardiovascular diseases are prevalent among individuals aged over 50 years, causing high morbidity and ranking first as a cause of death (Ma et al., 2021). In 2019, cardiovascular diseases accounted for approximately one-third of global deaths, with the highest number of deaths occurring in China (Roth et al., 2020). According to the 2022 *China Cardiovascular Health and Disease Report*, China currently has 13 million people with stroke, 11.39 million people with coronary heart disease, 8.9 million people with heart failure, 5 million people with cor pulmonale, 4.87 million people with atrial fibrillation, 2.5 million people with rheumatic heart disease, 2 million people with congenital heart disease, 45.3 million people with peripheral artery disease, and

245 million people with hypertension (The Writing Committee Of The Report On Cardivascular Health And Diseases and Hu, 2023). Neurological disorders are also widely prevalent. Neurological diseases were the second leading cause of death globally in 2015, resulting in approximately 9.4 million deaths, accounting for 16.8% of deaths. From 1990 to 2015, the number of deaths due to neurological diseases increased by 36.7% and disability-adjusted life years increased by 7.4% (Global Burden of Disease Study, 2013 Collaborators, 2015; Zhou et al., 2024). The high morbidity and mortality rates associated with these diseases urgently require effective treatment modalities to curb their progression (*Ginkgo biloba* L.). extract (GBE) has been approved for clinical use for treating various cardiovascular, metabolic, and neurodegenerative disorders (Peng et al., 2024). One of the critical components in GBE is flavonoids, which comprise up to 24% of its total content (Tao et al., 2022).

Amentoflavone (AME) is a flavonoid compound first isolated from the *Selaginella* plant (Okigawa et al., 1971). AME is one of the most common biflavonoid compounds found in *Ginkgo biloba* (Šamec et al., 2022). However, some studies suggest that the AME in *Ginkgo biloba* leaf extracts exhibits no biological activity and AME has been removed from the listing of the active components of such extracts (Xu et al., 2013). Recent research has demonstrated that AME has a wide range of pharmacological properties, including anti-inflammatory (Zhang and Wang, 2013), antioxidant (Li et al., 2020), anti-aging (Park and Kim, 2019), antibacterial (Bajpai et al., 2019), antiviral (Lee et al., 2023), anti-tumor (Qiu S. et al, 2021), antidepressant and anxiolytic (Ishola et al., 2012). AME also has beneficial effects on cardiocerebrovascular diseases and neurological diseases (Li et al., 2021; Saeedan et al., 2023; Sirimangkalakitti et al., 2019).

A bibliometric analysis was conducted on the literature pertaining to AME from 2014 to 2023, providing insights into the research advancements in the field on a global scale. The keyword "Amentoflavone" was queried in the Web of Science database, yielding a total of 394 research papers from 63 countries and 641 institutions, authored by 2,196 individuals. Figure 1A displays the countries with the highest number of publications, providing insight into the level of interest in AME among these nations. Analysis of the data indicates a consistent upward trend in publication output over the past decade, with the number of articles published in recent years significantly surpassing those published in 2014. This trend suggests sustained interest and advancement in the field of AME, as depicted in Figure 1B. Research on the role of AME in cardiocerebrovascular diseases and neurological disorders has gained increasing attention since 2015, emerging as a current research hotspot in the field. Furthermore, the impact of AME on other conditions such as tumors and diabetes should not be underestimated.

This review provides a comprehensive summary of the chemical structure and physicochemical properties of AME, encompassing its botanical sources, extraction and purification techniques, and administration routes. Additionally, it examines the pharmacokinetic characteristics of AME, alongside its therapeutic effects on cardiocerebrovascular diseases and neurological disorders, elucidating the underlying pharmacological mechanisms. The objective of this review is to enhance the understanding of AME and its potential applications in the treatment of cardiocerebrovascular diseases and neurological disorders.

2 Background

2.1 Chemical structure and physicochemical properties of AME

AME is a biflavonoid compound with the chemical name 8-[5-(5,7-dihydroxy-4-oxo-4H-chromen-2-yl)-2-hydroxyphenyl]-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one. It is an apigenin dimer, with multiple double bonds and hydroxyl groups in its molecular structure (Figure 2). The C_2-C_3 double bond is susceptible to hydrogenation, whereas the hydroxyl group is prone to substitution with methoxy groups. Therefore, many hydroxylated derivatives have an amentoflavone nucleus (Yu et al., 2017; Šamec et al., 2022; Xiao et al., 2018). It has been reported that the hydroxyl groups at positions C_7 and $C_{4'''}$ are crucial for the anti-inflammatory activity of AME. Substitution of these hydroxyl groups with methoxy groups significantly reduces the anti-inflammatory efficacy (Mangmool et al., 2024). Additionally, the atropisomerism exhibited due to the $C_{3'}$-$C_{8''}$ linkage in the structure of AME may affect its binding to targets, potentially influencing its biochemical activities (Bhattacharya and Mandal, 2024).

The molecular formula of AME is $C_{30}H_{18}O_{10}$, the molecular weight is 538.46 g/mol, and the melting point is 300°C (Xiong et al., 2021). The cross-conjugated molecular structure has strong characteristic absorption of ultraviolet light (Li, 2011). AME is a planar molecule with a close arrangement between molecules and large intermolecular attraction; therefore, it is poorly soluble in water, but is easily soluble in organic solvents such as ethanol and dimethyl sulfoxide (Xiong et al., 2021). Among the various polymorphs of AME, the amorphous form exhibits higher dissolution rates and solubility, and demonstrates good physical stability during the dissolution process (Zhou et al., 2022). It has been reported that the monomer apigenin from AME can interrupt free radical chain reactions and reduce the photoxidation process by generating resonance-stabilized free radicals (Huvaere et al., 2012). The presence of catechol structures and a double bond adjacent to the carbonyl group, along with hydroxyl groups on a single benzene ring, plays a crucial role in effectively quenching singlet oxygen (Nagai et al., 2005). This indirectly suggests that AME may exhibit strong antioxidant and photostability properties.

2.2 AME sources, and methods of extraction, separation, and purification

2.2.1 Sources

AME is a bioactive compound present in numerous plant species. It was initially isolated from the leaves of (*Selaginella tamariscina* Maxim.), (*Selaginella rupestris* L.), and *Ginkgo biloba*. Subsequently, it has been extracted from over 120 plants species, including (*Celaenodendron mexicanum* L.), (*Cupressus funebris* Endl.), (*Garcinia multiflora* Bl.), and (*Hypericum perforatum* L.) (Xiong et al., 2021). The *Selaginella* genus, comprising 21 species, is

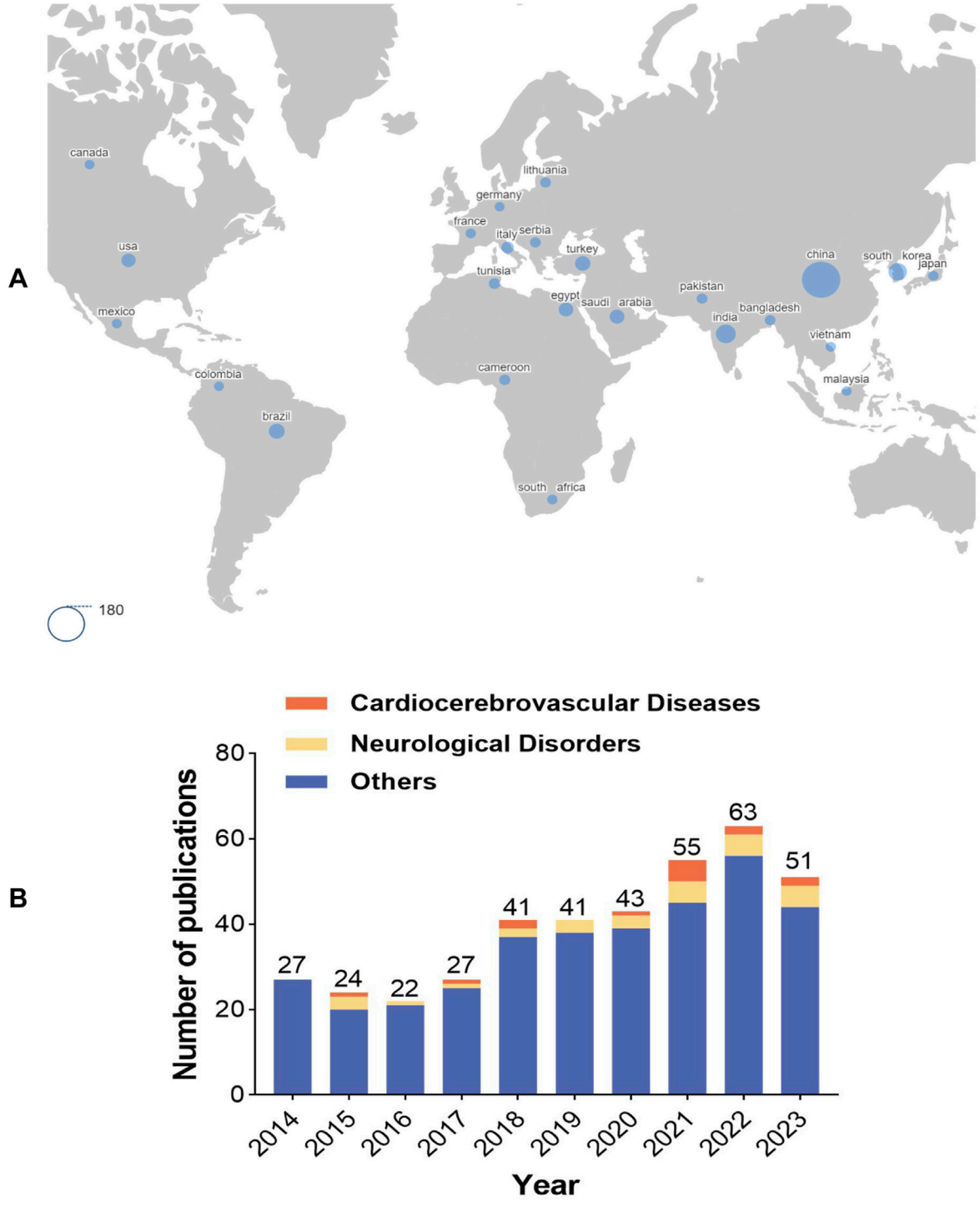

FIGURE 1
Bibliometric analysis of AME **(A)**. Global distribution map of countries involved in AME. The larger the blue circle in the figure is, the greater the number of articles published by the corresponding country. The area of the circle in the lower left corner is equivalent to 180 articles. **(B)**. Number of publications on AME in the past decade. Others represented other research fields of AME, with tumors and diabetes as the main research field.

the most prevalent source of amentoflavone. Other significant botanical families containing this compound include Cupressaceae, *Euphorbiaceae*, and *Clusiaceae*. Typically, AME is extracted from the leaves, aerial parts, and whole plants (Yu et al., 2017).

2.2.2 Extraction methods

The principal methods for the extraction of AME include ultrasonic-assisted extraction, microwave-assisted extraction, organic solvent extraction, and semi-bionic extraction (Table 1). Each of these methodologies presents distinct advantages and

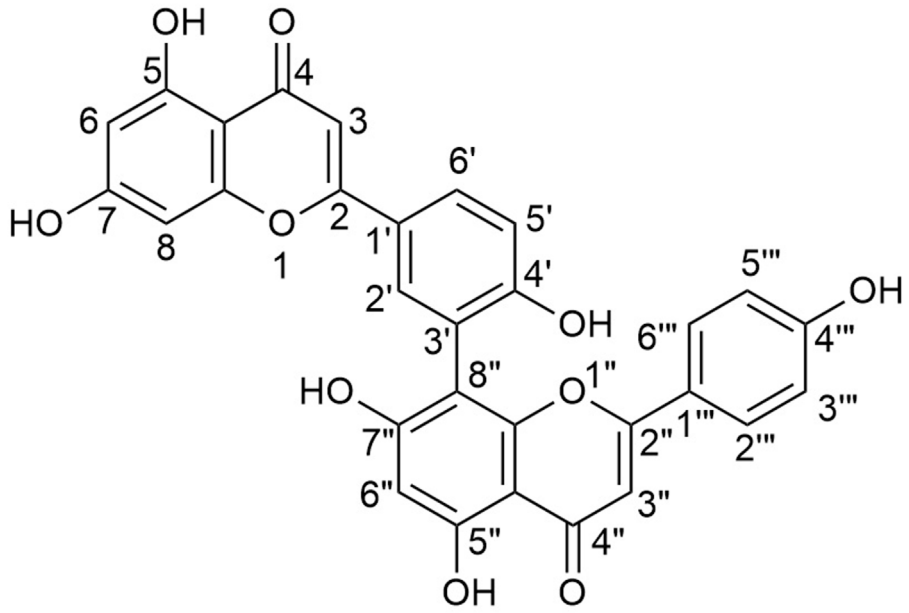

FIGURE 2
Chemical structure of amentoflavone (8-[5-(5,7-dihydroxy-4-oxo-4H-chromen-2-yl)-2-hydroxyphenyl]-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one).

TABLE 1 Methods of extracting amentoflavone.

Source	Extraction method	Extraction yield (%)	Reference
Selaginella tamariscina	Infrared-assisted extraction	0.290	Wang et al. (2018a)
Selaginella tamariscina	Ultrasonic-assisted ionic liquid extraction	1.351	Jiang et al. (2020)
Selaginella tamariscina	Ethanol reflux extraction	1.274	Wei (2010)
Selaginella pulvinata	Ethanol reflux extraction	1.728	Luo (2017)
Selaginella sinensis	Ionic liquid-microwave-based extraction	0.196	Li YY et al. (2019)
Selaginella moellendorffii	Deep eutectic solvent extraction	0.275	Liu et al. (2022)
Selaginella doederleinii	Ionic liquid-microwave-based extraction	0.650	Wang et al. (2018b)
Selaginella doederleinii	Microwave-assisted extraction	0.330	Wang et al. (2018c)
Selaginella uncinata	Ultrasonic-assisted extraction	1.530	Lai et al. (2018a)
Podocarpus nagi	Ethanol reflux extraction	0.010	Wang (2017)
Cunninghamia lanceolata	Ultrasonic-assisted extraction	0.319	Wang et al. (2023)
Taxus chinensis	Supercritical-CO_2 fluid extraction	0.447	Ruan et al. (2014)

disadvantages. For example, ultrasonic-assisted extraction offers benefits such as reduced extraction time, simplicity of operation, and high extraction efficiency. Nevertheless, its limited effective action area renders it unsuitable for industrial-scale production. Conversely, microwave-assisted extraction is noted for its straightforward operation, minimal byproduct formation, high extraction rates, and ease of product purification. However, it necessitates elevated extraction temperatures, which may compromise the integrity of active components. Organic solvent extraction is both cost-effective and straightforward; however, it suffers from low extraction efficiency, environmental pollution, and potential risks to human health make it less suitable (Li, 2011; Xu et al., 2021). Eutectic solvent (Liu et al., 2022) and infrared-assisted (Wang Y. et al., 2018) extraction techniques are gaining prominence in the extraction of AME due to their environmental sustainability, rapid processing times, and high efficiency.

2.2.3 Separation and purification methods

Currently, the primary methodologies for the separation and purification of AME are silica gel column chromatography, two-step precipitation, polyamide column chromatography, macroporous resin adsorption, and preparative high-performance liquid

TABLE 2 Methods of separating and purifying amentoflavone.

Source	Separation and purification method	Purity (%)	Recovery (%)	Reference
Selaginella tamariscina	Two-step precipitation method	58.2	88.7	Wei (2010)
Selaginella tamariscina	High-speed countercurrent chromatography	99.2	94.7	Wei (2010)
Selaginella tamariscina	Silica gel column chromatography	97.2	50.7	Wei (2010)
Selaginella tamariscina	Low pressure column chromatography	98.7	87.8	Wei (2010)
Selaginella pulvinata	Macroporous adsorption resin (HPD 300)	52.5	62.4	Luo (2017)
Selaginella pulvinata	Two-step precipitation method	47.9	59.7	Luo (2017)
Selaginella uncinata	Macroporous adsorption resin (NKA-9)	N/A	64.3	Lai et al. (2018b)
Selaginella moellendorffii	Macroporous adsorption resin (D-101)	80.8	62.5	Fang et al. (2011)
Podocarpus nagi	Macroporous adsorption resin (AB-8)	93.6	N/A	Wang (2017)

chromatography (HPLC) (Table 2). Researchers have been actively investigating enhanced techniques for separation and purification. Recently, flash chromatography has emerged as a novel method. Compared to traditional silica gel column chromatography, flash chromatography offers reduced the loading time, minimized dead adsorption, and improved efficiency and product purity. However, it exhibits lower separation capacity and is prone to interference from metal ions during elution. Macroporous resin adsorption offers several advantages, including rapid high-capacity and selective adsorption, as well as high elution efficiency, rendering it suitable for large-scale production. Nonetheless, its desorption efficiency remains suboptimal, and the purification rate is affected by the type of eluent and temperature (Xu et al., 2021). HPLC offers advantages including wide applicability, exceptional quantitative capabilities, and well-recognized methodologies. Nevertheless, it is marked by the necessity for extensive sample preparation, prolonged analysis durations, and expensive instrumentation (Vlasiou, 2023).

2.3 AME dosing forms

AME exhibits significant pharmaceutical potential and promise as a therapeutic agent (Xiong et al., 2021; Li et al., 2021). However, its poor water solubility constrains its release within the body, resulting in incomplete gastrointestinal absorption and low oral bioavailability, thereby limiting its pharmacological efficacy (Ren et al., 2013a). Following oral administration in rats, AME is dominantly distributed in the small intestine, stomach, liver, and large intestine, with minimal distribution to other tissues. To maximize the pharmacological efficacy of AME, it is imperative to enhance its solubility and bioavailability. Key strategies for improving drug solubility and bioavailability include modifying the delivery method, formulation (Table 3), and structural enhancement (Feng et al., 2020).

2.4 Pharmacokinetics of AME

Despite the multiple beneficial biological properties of AME, its pharmacokinetics remain inadequately characterized. A comprehensive understanding of AME's pharmacokinetics is crucial for elucidating its *in vivo* mechanisms of action, characterizing its properties, and optimizing drug design and dosing, to maximize therapeutic efficacy. Insights into the fundamental pharmacokinetics of AME can be inferred from studies conducted in animal models (Table 4).

It has been demonstrated that AME is absorbed via passive diffusion in rats (Wei et al., 2017). Furthermore, a study employing the Caco-2 cell model indicated that AME exhibits intestinal absorption. The absorption may involve paracellular passive diffusion and clathrin-mediated endocytosis, while the efflux transporter appears to be uninvolved (Wang B. et al., 2020). The low bioavailability of AME is due to extensive glucuronidation catalyzed by uridine diphosphate glucuronosyltransferase 1 family, polypeptide A (UGT1A1) and UGT1A3 (Gan et al., 2020).

Another study conducted demonstrated that the peak blood concentration in rats was reached at 90 min following oral administration, with a volume of distribution (V/F) of 198.36 ± 17.422 L/kg. These findings suggest that AME is predominant distributed or sequestered in specific tissues and organs within the rat model. (Wang et al., 2015).

In vivo, 34 metabolites of AME were identified, while 24 metabolites were identified *in vitro*. In the *in vivo* study, all metabolites were distributed in feces, with three of the 34 metabolites were also detected in urine, and none were found in bile or plasma. In the *in vitro* study, 20 of the 24 metabolites were distributed in liver microsomes, and 17 of the 24 metabolites were associated with the intestinal microbiota. Of the total metabolites identified both *in vivo* and *in vitro*, 14 were classified as phase I metabolites and 26 as phase II metabolites. The primary metabolic pathways included oxidation, methylation, acetylation, and oxidation methylation (Feng, 2020). It has been reported that the bioactive form of AME is likely conjugated (Liao et al., 2015). Another study found that 90.7% of AME circulates as conjugated metabolites post-administration. In rats, 73.2% and 70.2% of AME in plasma were in conjugated form following intravenous and intraperitoneal injection, respectively (Yu et al., 2017).

The cumulative excretion rates of AME in feces and urine were 23.93% and 0.82%, respectively, indicating that fecal excretion is the predominant route of AME elimination from the body. This high rate of fecal excretion may contribute to the compound's low bioavailability (Chen et al., 2022).

TABLE 3 Different formulations to improve amentoflavone.

Route of administration	Formulation	Particle size	Zeta potential	Improved results	Reference
Oral	Micro-emulsion formulation	15.37 ± 0.09 nm	−17.1 ± 0.24mV	Dissolution rate	Ren et al. (2013a)
Oral	Micro-powder formulation	0.08 ± 0.01 μm	N/A	Solubility, dissolution rate	Ren et al. (2013b)
Intravenous	Nanoparticulate formulation	77.3 ± 5.3 nm	−2.92 ± 0.27 mV	Solubility, dispersibility, stability, bioavailability, reduce toxicity	Zhao et al. (2022)
Oral	Micelle formulation	58.8 ± 1.29 nm	5.26 ± 0.63 mV	Bioavailability	Zhang et al. (2019)
Oral	Micelle formulation	67.33 ± 2.01 nm	−0.84 ± 0.04 mV	Bioavailability	Feng et al. (2020)
Oral	Sub-micron particle formulation	Approximately 0.4 μm	N/A	Solubility, dispersibility, stability, dissolution rate	Duan et al. (2022)
Oral	Micelle formulation	25.99 ± 0.10 nm	−27.67 ± 0.25 mV	Solubility, dissolution rate; Bioavailability	Feng et al. (2023)
Intranasal	Nanoemulsion formulation	Approximately 37 nm	Approximately −4 mV	Bioavailability	Khafagy et al. (2023)

3 Biological activity of AME for treating cardiocerebrovascular and neurological diseases

Atherosclerosis is a main pathological basis for cardiovascular and cerebrovascular diseases (Dutta et al., 2023). The cerebrovascular system is closely related to the structure and function of brain tissue. Vascular factors are important in the development of neurological diseases (Iadecola, 2023; Smith et al., 2021). Therefore, this section discusses the effect of AME on cardiocerebrovascular and neurological diseases (Figure 3).

3.1 Potential mechanisms of AME in atherosclerosis

Although there is currently no published literature regarding the use of AME in the treatment of atherosclerosis, its various potential mechanisms of action are promising. The process of atherosclerotic plaque formation can be divided into the following steps (Figure 4): (1) When the vascular wall is damaged, low-density lipoprotein (LDL) enters the intimal layer of the blood vessel through the gaps between endothelial cells (ECs), forming oxidized low-density lipoprotein (ox-LDL). Monocytes migrate into the intimal layer and are activated into macrophages. (2) Ox-LDL binds to receptors on the surface of macrophages, and cholesterol enters the macrophages, where it is esterified. When the intake, esterification, and release of cholesterol are out of equilibrium, intracellular lipid overload leads to the formation of macrophage-derived foam cells, creating fatty streaks in the lesions (Gui et al., 2022). (3) Ox-LDL induces changes in the phenotype of vascular smooth muscle cells (VSMCs) in the tunica media of the arterial wall, leading to abnormal proliferation and migration to the intimal layer. Subsequently, VSMCs engulf ox-LDL to form myogenic foam cells (Yang, 2023; He, 2013), which then form a fibrous plaque. (4) Necrosis and disintegration of macrophage-derived and myogenic foam cells lead to atherosclerotic plaque formation. Inflammatory cells secrete matrix metalloproteinases (MMPs) to break down collagen fibers in the extracellular matrix, thereby increasing plaque instability. Subsequently, the plaque ruptures, causing bleeding and thrombosis (Carracedo et al., 2019; Libby, 2021; Poznyak et al., 2020). Atherosclerosis is a chronic inflammatory disease involving various inflammatory, free radical, and oxidative stress-related injuries (Meng et al., 2024).

3.1.1 Inhibiting inflammatory factors

When inflammation occurs *in vivo*, IκB kinase phosphorylates inhibitor of NF-κB (IκB) protein, and then IκB protein is separated from the p50 and p65 subunits of nuclear factor-kappa B (NF-κB), so that NF-κB is activated and enters the nucleus for gene transcription and expression of inflammatory factors, inflammatory mediators, chemokines, and adhesion factors (Guo et al., 2024). AME can protect vascular ECs through various mechanisms. AME increases the survival of human umbilical vein endothelial cells (HUVECs) induced by TNF-α in the S phase of cell proliferation. Disruption of the endothelin-1 (ET-1)/nitric oxide (NO) balance in the blood is an indicator of vascular endothelial damage. AME can increase the NO content of HUVECs, reduce the level of ET-1, inhibit the expression of adhesion factors vascular cell adhesion molecule-1 and E-selectin, and inflammatory factors interleukin IL-6 and IL-8, enhance the expression of the inhibitory protein IκBα activated by NF-κB, and reduce the expression of its transcription factor NF-κB in the nucleus, preventing further damage to the vascular endothelium (Zheng et al., 2013). AME downregulates the release of NO from mouse macrophages stimulated by lipopolysaccharide (LPS), and the levels of TNF-α and IL-1β induced by LPS and monosodium urate in THP-1 macrophages, via the NOD-like receptor thermal protein domain associated protein 3 (NLRP3)/apoptosis-associated speck-like protein/cysteinyl aspartate specific protease (caspase)-1 signaling pathway (Zhang X. et al., 2021). An experiment was conducted to examine the binding effects of 34 flavonoid products on the NLRP3 inflammasome, using CB-Dock molecular docking for binding predictions. It was found that AME has the strongest

TABLE 4 Pharmacokinetic parameters of amentoflavone.

Route of administration	Dose	C_{max}	T_{max}	$T_{1/2}$	AUC_{0-t}	$AUC_{0-\infty}$	*CL/F*	*V/F*	MRT_{0-t}	$MRT_{0-\infty}$	*F* (%)	Reference
Oral	2.8 g/kg	22.5 ± 1.4 ng/mL	1.13 ± 0.44 h	2.06 ± 0.13 h	125 ± 7 ng/h mL	133 ± 8 ng/mL h	N/A	N/A	N/A	N/A	N/A	Wang et al. (2014)
Intragastric	60 mg/kg	0.469 ± 0.046 min/L	90.0 min	57.01 ± 2.765 min	27.7 ± 1.1 mg/L min	24.9 ± 1.1 mg/L min	2.4 ± 0.1 L min^{-1} kg^{-1}	198.4 ± 17.4 L/kg	122.8 ± 1.6 min	126.1 ± 2.3 min	N/A	Wang et al. (2015)
Oral	500 mg/kg	42.37 ± 11.95 ng/mL	0.85 ± 0.137 h	12.33 ± 4.65 h	194.5 ± 16.9 ng/mL h	299.2 ± 75.4 ng/mL h	N/A	N/A	9.58 ± 0.84 h	N/A	0.06 ± 0.04	Gan et al. (2020)
Intravenous	10 mg/kg	17,505 ± 1,532 ng/mL	0.033 h	9.36 ± 2.97 h	10,060.9 ± 1,163.8 ng/mL h	10,706.6 ± 1,225.9 ng/mL h	N/A	N/A	3.365 ± 0.34 h	N/A	N/A	Gan et al. (2020)
Intravenous	10 mg/kg	31.2 ± 27.0 nmol/mL	0.11 ± 0.02 h	5.88 ± 1.78 h	33.0 ± 11.9 nmol/mL h	35.2 ± 13.9 nmol/mL h	320 ± 139 mL h^{-1} kg^{-1}	2.53 ± 0.65 L/kg	N/A	N/A	N/A	Liao et al. (2015)
Intraperitoneal	10 mg/kg	6.26 ± 0.33 nmol/mL	0.83 ± 0.29 h	3.42 ± 1.45 h	25.5 ± 1.08 nmol/mL h	25.7 ± 0.82 nmol/mL h	N/A	N/A	N/A	N/A	77.4 ± 28.0	Liao et al. (2015)
Oral	300 mg/kg	0.06 ± 0.03 nmol/mL	0.33 ± 0.14 h	11.3 ± 3.6 h	0.41 ± 0.08 nmol/mL h	0.49 ± 0.12 nmol/mL h	N/A	N/A	N/A	N/A	0.04 ± 0.01	Liao et al. (2015)
Oral	4.31 mg/kg	124.61 ± 8.37 ng/mL	1.5 h	2.60 ± 1.34 h	594.48 ± 62.12 μg h/L	597.84 ± 60.41 μg h/L	7.27 ± 0.75 L min^{-1} kg^{-1}	N/A	N/A	N/A	N/A	Shan et al. (2018)

C_{max}, maximum blood concentration; T_{max}, time to peak concentration; $T_{1/2}$, biological half-life; AUC(0-t), area under the concentration-time cure; AUC(0-∞), from time zero to all original drug elimination; *CL/F*, clearance; *V/F*, apparent volume of distribution; $MRT_{0-\infty}$, mean residence time; MRT_{0-t}, average retention time for a certain period of time; *F*, bioavailability.

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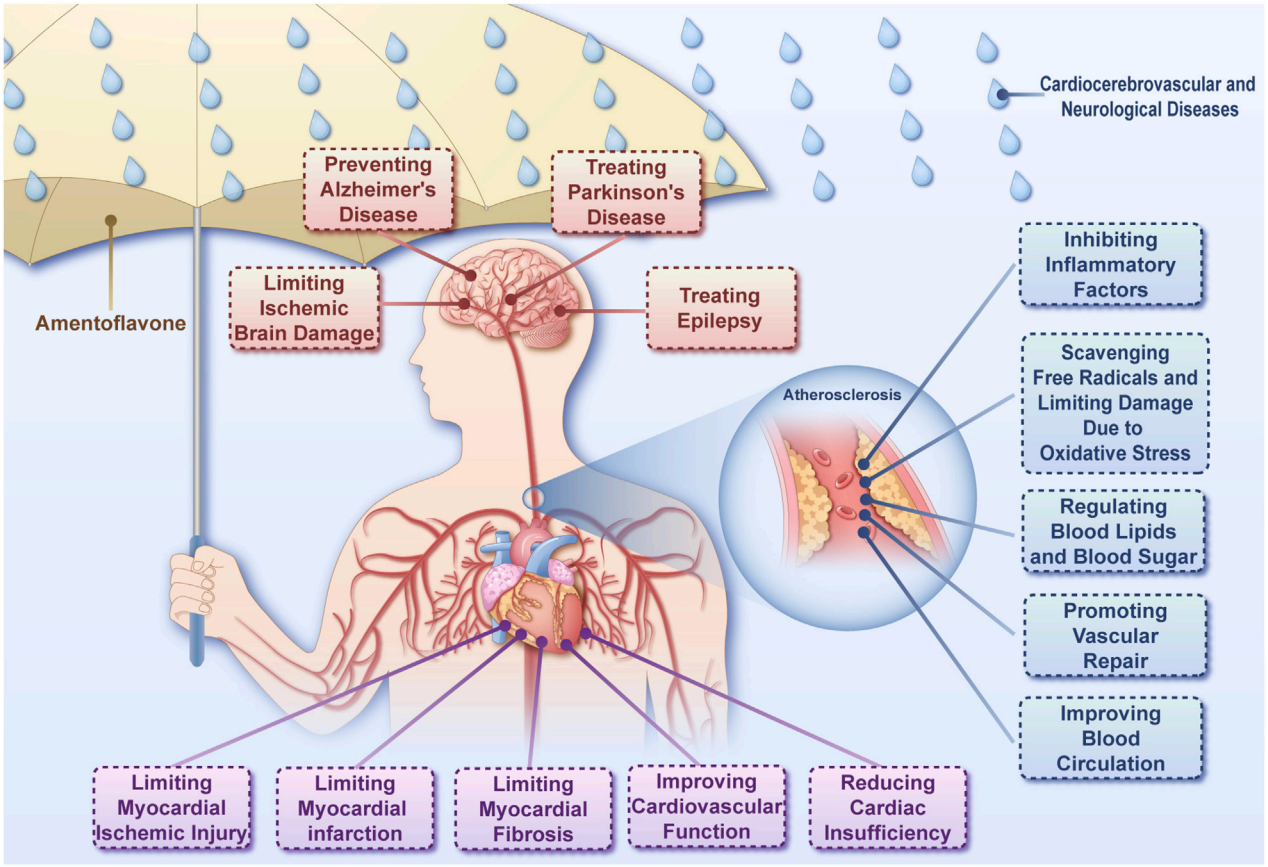

FIGURE 3
The ameliorative effect of amentoflavone on cardiocerebrovascular and neurological diseases Amentoflavone is capable of acting on cardiocerebrovascular diseases, neurological diseases through a variety of mechanisms.

affinity for the NLRP3 inflammasome, surpassing that of its specific inhibitor (CY-09) (Fang H. Y. et al., 2023). Macrophage migration inhibitory factor (MIF) is a crucial pro-inflammatory mediator. AME has been reported to exhibit superior inhibitory activity against MIF compared to ISO-1, a well-established standard MIF inhibitor (Siddiqui et al., 2024).

During inflammation, arachidonic acid is converted to prostaglandin E−2 (PGE-2) by cyclooxygenase-2 (COX-2) through the action of cyclooxygenase-2, leading to pain and inflammation. At a concentration of 50 μM, AME can inhibit the activity and expression of COX-2 induced by TNF-α in cells, and upregulate the activity of peroxisome proliferator-activated receptor (PPAR) γ, blocking the degradation of IκBα, and inhibiting the translocation of NF-κB to inhibit the activation of the NF-κB signaling pathway (Banerjee et al., 2002). AME isolated from the root of (*Prismatomeris glabra* Mart.) exerts an anti-inflammatory effect by significantly reducing the production of TNF-α, IL-6, and PGE-2 in THP-1-derived macrophages in a dose-dependent manner (Alkadi et al., 2021). AME effectively suppresses the production of NO and PGE-2 in RAW264.7 macrophage cells stimulated by LPS. This inhibitory effect is achieved by inhibiting the kinase activity of extracellular signal-regulated kinase (ERK). Furthermore, AME also inhibits the expression of inflammation-related genes induced by LPS, including nitric oxide synthase (iNOS), COX-2, and TNF-α (Oh et al., 2013).

Macrophages of different polarization types are involved in distinct atherosclerosis development processes. Specifically, M1-type macrophages are primarily present in early plaques, associated with plaque formation. M2-type macrophages can exert anti-inflammatory effects, promoting the repair of atherosclerosis inflammation. PPARs are a class of ligand-activated transcription factors that play a crucial role in macrophage polarization, regulating macrophage metabolism, suppressing pro-inflammatory genes, and promoting the transformation of M2 macrophage phenotype (Zhuang, 2020). PPARγ, through its interaction with other transcription factors and the promoter regions of arginase 1 (*Arg1*), found in inflammatory zone (*Fizz1*), and chitinase-like protein 3 (*Ym1*) genes, promotes the expression of *Arg1* and *Fizz1* genes and regulates the M2 polarization level of macrophages. Macrophages lacking PPARγ exhibit significantly downregulated expression of *Arg1*. AME can inhibit the differentiation of THP-1-derived M0 cells toward M1 cells by activating PPAR-α/γ transcription factors, elevating the mRNA levels of TGF-β and IL-10, reducing TNF-α and IL-6 expression, and upregulating *Arg1* and *Fizz1* protein expression (Qiu F. et al., 2021). These findings suggest that AME

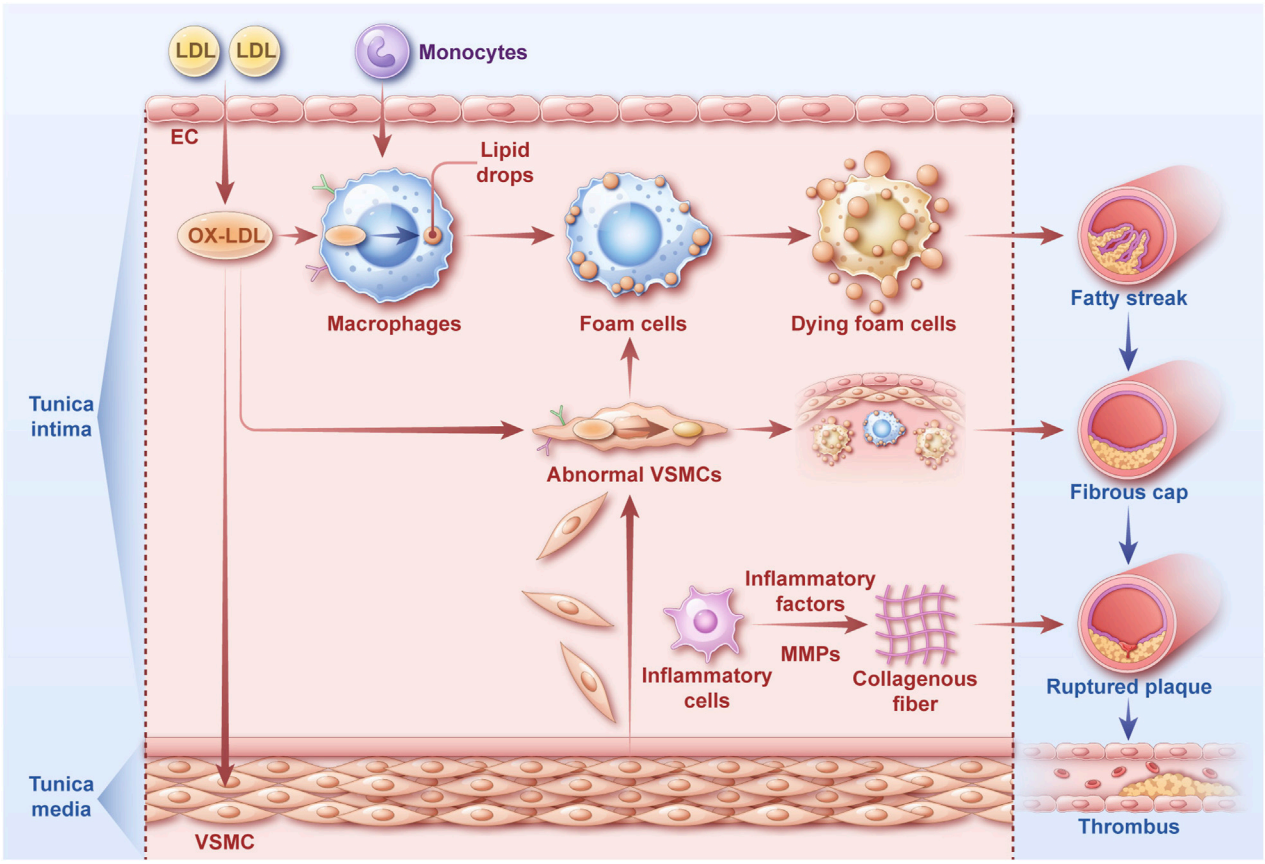

FIGURE 4
The atherosclerotic process The key steps of the atherosclerotic process can be divided into the following steps: Formation of oxidized low-density lipoprotein and activation of macrophages; Macrophage-derived foam cell formation; Abnormal VSMCs and myogenic foam cell formation; Formation and rupture of atherosclerotic plaque.

might also reverse the transformation of M1 macrophages toward the M2 type, thereby exerting an anti-inflammatory effect.

3.1.2 Scavenging free radicals and limiting damage due to oxidative stress

NO produced iNOS in activated macrophages is one of the most important inflammatory mediators. iNOS-mediated NO production and the associated production of highly reactive free radicals such as peroxynitrite has a harmful effect (Wang Y. et al., 2020). Therefore, inhibiting NO may be a useful target for addressing oxidative stress.

In vitro antioxidant models have demonstrated that AME exhibits exceptional scavenging and antioxidant capabilities when eliminating (1,1-diphenyl-2 trinitrophenylhydrazine) DPPH free radicals, superoxide anions, and hydroxyl radicals. Additionally, AME possesses the ability to repair and protect DNA from oxidative damage *in vitro*. AME influences the generation and scavenging of OH free radicals, and eliminates free radicals by scavenging hydrogen ion and electron. Therefore AME can treat oxidative damage (Wang, 2013). AME can eliminate DPPH free radicals in a concentration-dependent manner and can alleviate the cell damage caused by ox-LDL in HUVECs. The mechanism might be due to the phenolic hydroxyl group in the molecular structure of AME, which can accept the electron transfer from lipid peroxidation free radicals, forming stable free radicals, thus preventing the damage of lipid peroxidation free radicals to vascular ECs (Xu et al., 2004). Advanced glycation end-products (AGEs) are associated with various diseases such as atherosclerosis and diabetes, leading to the production of reactive oxygen species (ROS), which subsequently activates the transcription factor NF-κB and is involved in various inflammatory diseases. Ferchichi et al. extracted various active components from plants, and showed that AME had the strongest *in vitro* ability to effectively resist AGEs and exhibits excellent scavenging capabilities (Ferchichi et al., 2012). Furthermore, AME can block the nuclear translocation of NF-κB p65, inhibit the phosphorylation of IκBα and formation of NO in macrophages induced by LPS in a concentration-dependent manner by blocking the degradation of IκBα to inhibit the formation and transcription activation of the iNOS gene induced by LPS (Woo et al., 2005).

AME reduces LPS-induced oxidative stress damage to HUVECs, increasing the activity of superoxide dismutase (SOD) and reducing the expression levels of NO and malondialdehyde (MDA). A multi-omics study found that glycine, argininosuccinic acid, putrescine, ornithine, spermidine, 5-oxoproline, and dihydrouracil are seven metabolites that might be related to the mechanism by which AME protects ECs (Yao et al., 2016).

The reduced form of thioredoxin (Trx) interacts with the N-terminus of apoptosis signal regulating kinase 1 (ASK1) both

in vitro and *in vivo*, thereby inhibiting ASK1 activity. Under conditions of oxidative stress, the thiol group of the cysteine residue of Trx is oxidized to form intramolecular disulfide bonds, thereby activating ASK1 kinase activity. Therefore, in the complex of Trx with ASK1 protein, Trx1 and thioredoxin reductase (TrxR)-1 proteins are the key molecules regulating ROS-induced ASK1 activation. AME can regulate the ROS/ASK1/p38 mitogen-activated protein kinase (MAPK) pathway by inactivating ASK1 molecules, blocking p38 MAPK signaling, increasing the levels of thioredoxin Trx1 and reductase TrxR-1, and reducing ASK1 and p38 MAPK levels, thereby reducing oxidative stress damage to cells (Li et al., 2020). These studies demonstrate that AME can respond to oxidative stress through various mechanisms.

3.1.3 Regulating blood lipids and blood sugar

The fat accumulation index can serve as a predictive indicator of cardiometabolic diseases and stroke. The fat accumulation index is linearly related to the risk of atherosclerotic cardiovascular disease within 10 years and is an independent risk factor, indicating an integral relationship between body fat and atherosclerotic diseases (Liu et al., 2023). A randomized trial involving over 20 million participants demonstrated that blood lipid components such as LDL, apolipoprotein A, apolipoprotein B, and triacylglycerols can also affect the development of atherosclerosis (Ference et al., 2017). Therefore, controlling the level of blood lipids can alleviate and prevent atherosclerosis.

Obesity leads to the storage of excess energy in the form of triglycerides (TG) in adipose tissue. Dietary fat can lead to increased TG levels in the blood and obesity, whereas lipid absorption disorders can lead to hyperlipidemia and metabolic diseases. The cluster of differentiation 36 (CD36) protein primarily participates in the process of macrophage lipid uptake. When a large amount of ox-LDL appears in the vascular endothelial layer, the CD36 protein is activated to take up ox-LDL into the cell. After intake, ox-LDL is oxidized to new derivatives by linoleic acid, and these new derivatives can activate PPARγ, thereby promoting the increase in PPARγ protein expression as a transcription factor of CD36. Increased CD36 expression in turn promotes ox-LDL uptake. AME can reduce the uptake of ox-LDL by THP-1-derived macrophages through the CD36/PPARγ signaling pathway, thereby inhibiting foam formation induced by ox-LDL (Zhuang, 2020). Feeding AME to mice on a high-fat diet reduced the expression of the lipid absorption-related gene, CD36, by affecting plasma TG levels, thereby affecting the intestinal absorption of lipids (Lee et al., 2022). AME can reduce the body weight, total fat tissue, and serum TG content induced by a high-fat diet in a dose-dependent manner. AME has been demonstrated to reduce blood glucose levels and insulin resistance in rat models. Furthermore, AME influences various stages of 3T3-L1 adipocyte differentiation. Specifically, it modulates ROS production and inhibits the expression of the transcription factor CCAAT/enhancer-binding protein (C/EBP)-β during the mitotic clonal expansion (MCE) phase, thereby impacting MCE. Additionally, AME downregulates the expression of PPARγ and C/EBP-α during both the early and terminal differentiation phases, consequently affecting lipid droplet formation. (Chen et al., 2016).

Type 2 diabetes is intricately linked to atherosclerosis. Patients with diabetes often have disorders of lipid metabolism and insulin resistance syndrome. Elevation of blood glucose and lipid levels in patients with type 2 diabetes are risk factors for atherosclerosis, and are positively correlated with the extent of atherosclerosis lesions (Sun et al., 2023; Huang and Sun, 2023). High blood glucose levels disrupt ECs function, causing damage the vascular wall, thereby promoting atherosclerosis progression (Wei and Liang, 2021). AME significantly suppresses the elevation of blood glucose levels and reduces the Homeostatic Model Assessment of Insulin Resistance index. Furthermore, they demonstrated that AME also reduced lipid accumulation in the liver of high fructose and fat diet (HFFD)-fed rats and alleviated the damage caused by lipids to the liver (Qin et al., 2018). AME reduced the expression of genes related to fat production and upregulated the expression of genes related to insulin signaling transmission, thereby having anti-obesity and anti-hyperglycemic effects (Cho et al., 2021).

In vitro hypoglycemic effects of AME using HepG2 cell glucose models and insulin resistance models and found that AME significantly increased glucose metabolism of HepG2 cells and had a synergistic effect on the increased glucose consumption under insulin stimulation. In the insulin resistance HepG2 cell model induced by high insulin, AME also increased the glucose consumption of cells that developed insulin resistance (Zheng et al., 2008). These characteristics suggest that AME may serve as an insulin sensitizer, increasing cell glucose consumption and synergistically working with insulin to reduce blood glucose levels and reduces insulin resistance. In the insulin resistance HepG2 cell model induced by high glucose and high insulin, AME significantly increased the levels of rate-limiting enzymes for glucose oxidative decomposition, such as 6-phosphogluconate kinase, glucose kinase, and pyruvate kinase; reduced glucose synthesis by lowering glucose synthesis kinase-3-β (GSK-3-β) levels; and lowered phosphoenolpyruvate carboxy kinase and glucose-6-phosphate enzyme activity, thereby affecting the glucose biosynthesis pathway. They also demonstrated that AME increases phosphoinositide 3-kinase (PI3K) protein expression, which may improve insulin signal transduction disorders through the PI3K/protein kinase B (Akt) signaling pathway, thereby alleviating insulin resistance (Ke et al., 2013). In type 2 diabetes model (T2DM) rats, AME reduces the release of TNF-α, upregulates glucose transporter 2 expression, enhances the absorption and utilization of blood glucose by the liver and skeletal muscle, and reduces insulin resistance through the PI3K/Akt/mechanistic target of rapamycin (mTOR) and PPARγ signaling pathways (Zhang et al., 2019).

At a dose of 60 mg/kg AME can repair damaged pancreatic tissues in mice with diabetes and enhanced pancreatic islet β cell function (Zheng et al., 2008). The molecular structure of AME can stably bind to the structure of human islet amyloid polypeptide (hIAPP), thereby interfering with the peptide assembly and abnormal folding process, separating hIAPP fibrils into small oligomers and particles, and reducing the cytotoxicity induced by hIAPP oligomerization (Xu et al., 2022). These results indicate that AME can regulate blood lipids and blood glucose from multiple perspectives and different mechanisms and can alleviate various injuries.

3.1.4 Promoting vascular repair

Endothelial growth factor (VEGF) is a key factor in the early formation of blood vessels and a highly selective mitogen for ECs, promoting the proliferation and migration of ECs to promote

angiogenesis (Wiszniak and Schwarz, 2021). AME promotes the proliferation of HUVECs in the mitotic S phase in a dose-dependent manner, and upregulates the expression of VEGF, indicating that it plays a role in repairing vascular endothelial cell damage (Zheng et al., 2011). However, under pathological conditions, VEGF increases the instability of atherosclerotic plaques by promoting angiogenesis and inflammatory infiltration, leading to plaque shedding (Qin et al., 2021). Using two different experimental models, it was found that AME can specifically bind to members of the VEGF family, VEGF-A and placental growth factor-1 (PIGF-1), preventing them from further binding to their receptors and inhibiting endothelial cell migration and capillary-like tube production induced by VEGF-A and PIGF-1, thereby inhibiting the growth and formation of vessels (Tarallo et al., 2011). The abnormal proliferation and migration of VSMCs can lead to vascular lesions, which are the hallmark of atherosclerosis, vascular intimal hyperplasia, and arterial stenosis. AME can inhibit the ox-LDL-induced transformation of VSMCs to foam cells through the CD-36/PPARγ signaling pathway, and can inhibit VSMC migration, thereby promoting vascular repair (Zhuang, 2020).

3.1.5 Improving blood circulation

The vasodilator effect of AME may be associated with the muscarinic receptor, the β-adrenergic receptor, and the endothelium-derived vasodilator factor. NO is produced by the catalytic action of iNOS on L-arginine. The vasodilator effect of NO is mediated through the increase of guanosine 3′,5′-cyclic monophosphate (cGMP) levels in smooth muscle. When the vascular endothelium experiences dysfunction, the release of NO decreases, and the vasoconstriction produced by directly activating vascular smooth muscle further reduces the production of NO (Borow et al., 2015). AME has a significant vasodilator effect; however, after injury to the vascular endothelium of injured rats, its vasodilator effect is inhibited, suggesting that AME may act on the vascular endothelium. The vasodilator effect of AME is also inhibited by NO inhibitors in intact vascular endothelium but is not affected by the addition of propranolol hydrochloride and atropine, suggesting that AME may produce a vasodilator effect by affecting the release of NO from the vascular endothelium (Xun and Yin, 2009). iNOS inhibitors can block the vasodilator effect of AME. Guanylate cyclase inhibitors also block the vasodilator effect of AME. Based on a series of experiments, they concluded that AME activates the Ca^{2+}-dependent K^{+} channel of ECs, affecting the NO-cGMP signaling pathway, thereby relaxing the vascular smooth muscle and causing vasodilation (Kang et al., 2004).

After the rupture of atherosclerotic plaques, the activation of platelets and thrombin promotes thrombus formation, leading to vascular blockage and interruption of circulation (Ahmed et al., 2020). Thrombin is a serine protease that plays a significant role in the coagulation cascade, thrombus formation, and platelet activation (Brummel et al., 2002). Therefore, drugs that act on thrombin can alleviate disease progression caused by atherosclerosis. AME in (*St. John's Wort* Diosc.) can inhibit the activity of human thrombin in a dose-dependent manner (Wei et al., 2019). The study compared the effects of 16 major components in *Ginkgo biloba* on thrombin and found that AME has a high affinity for human thrombin and is a strong human thrombin inhibitor. Subsequent molecular docking experiments revealed that it can primarily bind to key amino acids in the active site of thrombin through salt bridges and hydrogen bonds (Chen et al., 2019). Using an acute rat blood stasis model, it was demonstrated that AME can reduce plasma fibrinogen levels to prolong coagulation time, thereby improving blood circulation (Xi et al., 2020). Furthermore, AME has been shown to inhibit platelet aggregation induced by adenosine diphosphate and arachidonic acid, but has no effect on platelet aggregation induced by thrombin (Zhang et al., 2018). The mechanism underlying these effects warrant further study. These results suggest that AME could serve as a natural drug for targeting thrombin and platelets.

3.2 Effect of AME on cardiocerebrovascular diseases

3.2.1 Limiting myocardial ischemic injury, myocardial infarction and myocardial fibrosis

Myocardial ischemic injury can lead to myocardial infarction, and myocardial fibrosis may occur after infarction, a process that further affects heart function. If left untreated, myocardial ischemia can lead to damage of the heart, ultimately increasing the risk of developing myocardial infarction over time. Patients who have had a myocardial ischemia often have persistently elevated TNF-α levels. (Ridker et al., 2000). Overexpression of TNF-α, IL-1β, and IL-6 can amplify the harmful effects of inflammation by inducing cell apoptosis. Therefore, inhibiting these inflammatory factors is a promising strategy to prevent the escalation of myocardial ischemic injury. (Kumari et al., 2024).

Ischemic reperfusion injury experiments in mice, have shown that AME significantly reduces the levels of serum myocardial enzymes, specifically lactate dehydrogenase and creatine kinase MS isoenzyme, indicating the protective effects of AME on H9c2 myocardial cells. AME was also shown to significantly reduce the levels of IL-1β, IL-6, and TNF-α in cell supernatants, inhibiting cell apoptosis after myocardial ischemia-reperfusion injury in rats and reducing the size of the myocardial infarction area (Li et al., 2021). It was discovered that AME improved myocardial ischemia-reperfusion injury by modulating the PI3K/Akt-NF-κB signaling pathway, enhancing the phosphorylation of Akt and inhibiting the phosphorylation of NF-κB, reducing cardiac cell apoptosis, and lowering the release of associated inflammatory factors (Li, 2020). In the myocardial infarction model, AME can reduce the values of left ventricular end-systolic diameter and left ventricular end-diastolic diameter, and increase the value of left ventricular ejection fraction, indicating that AME can effectively improve the cardiac function of rats after myocardial infarction. AME can also reduce the expression of carboxy terminal peptide of type I procollagen and the amino terminal peptide of type III procollagen in rats after myocardial infarction, indicating that AME can inhibit myocardial fibrosis after myocardial infarction. The mechanism may involve downregulation of matrix metalloproteinase MMP-2 and transforming growth factor TGF-β1 expression (Chen et al., 2023).

3.2.2 Reducing cardiac insufficiency

AME can improve adriamycin (DOX)-induced cardiac dysfunction and reduce myocardial injury. Furthermore, AME

can significantly reduce the expression of cell pyroptosis-related proteins, such as NLRP3, cleaved caspase-1, and cleaved gasdermin D, thereby inhibiting myocardial cell pyroptosis without affecting the expression of apoptosis-related proteins. The primary mechanism of AME action is through the inhibition of the stimulator of interferon genes (STING)/NLRP3 inflammasome signaling pathway (Fang G. et al., 2023). The effect of AME on DOX-induced cardiotoxicity was investigated from several perspectives, including pathological characterization, antioxidant stress, mitochondrial function recovery, anti-inflammatory effects, and apoptosis inhibition. It was found that AME increases heart weight to reverse DOX-induced heart atrophy, ameliorates oxidative stress-induced cardiac injury by reducing MDA, inhibits NADPH oxidase (NOX) expression, increases SOD levels, upregulates the expression of myocardial mitochondrial-related genes nuclear respiratory factor-1 (*NRF-1*) and mitochondrial transcription factor A (*TFAM*) to address mitochondrial dysfunction, decreases IL-6 and NF-κB expression to exert anti-inflammatory effects, upregulates heat shock protein 27 (HSP-27) and downregulates fas ligand (Fasl) expression to influence the process of cell apoptosis (Alherz et al., 2022). Through comparing effects of different dietary supplements on rat atria, it was discovered that AME and quercetin might be the pharmacologically active components of GBE in terms of its positive inotropic and chronotropic effects. Moreover, 10–50 μg/mL of AME can significantly increase the heart rate of rat atria without altering myocardial contractility (Kubota et al., 2002). However, other studies have shown that AME can inhibit the activity of cAMP diesterase, thereby enhancing myocardial contractility and dilating peripheral vessels, further promoting blood flow in the body and reducing pressure on the heart and vessels (Saponara and Bosisio, 1998). This suggests that AME is a potential drug for the treatment of heart failure.

3.2.3 Improving cardiovascular function

It was discovered that AME exerts a protective effect on cardiovascular dysfunction through several mechanisms: (1) Ultrasonic electrocardiographic evaluations have shown that AME can inhibit the increase in left ventricular internal diameter and the thickness of the posterior wall during diastole, reduce left ventricular mass, alter the ejection fraction and relative wall thickness, and inhibit the increase in cardiac stiffness and left ventricular wet weight induced by an HFFD. (2) In settings of oxidative stress, AME can modify the levels of oxidative stress markers such as thiobarbituric acid reactive substances, glutathione (GSH), SOD, catalase (CAT) in plasma, and can inhibit the increase in NOX in the heart, thereby reducing the degree of oxidative stress-induced cardiac injury. (3) In rats fed a HFFD, AME inhibits the increased expression of angiotensin (Ang) II receptors, angiotensin-1A receptor and the decreased expression of angiotensin type 2 receptors in the renin-angiotensin system and can significantly inhibit the increase in blood pressure. (4) AME inhibits phenylephrine-induced aortic vasoconstriction and increased acetylcholine-induced vascular relaxation. The mechanism of action of AME may involve the regulation of NOX, thereby modulating angiotensin II cell signaling and oxidative stress (Qin et al., 2018).

3.2.4 Limiting ischemic brain damage

Ischemic stroke is one of the most common brain diseases, accounting for 85% of cerebrovascular diseases (Oliveira et al., 2023). Research has shown that AME can protect the brain from hypoxic-ischemic injury from multiple perspectives. First, administration of AME to rats with hypoxic-ischemic brain injury reduced brain tissue damage in the forebrain by 50%. Second, *in vitro* experiments showed that AME exhibits excellent neuroprotective effects against DNA damage, mitochondrial damage, and NO-induced injury. *In vivo* experiments demonstrated that AME inhibits caspase 3-induced cell pyroptosis in a dose-dependent manner, decreases the expression of iNOS and COX-2, and suppresses the production of inflammatory factors such as IL-1β and TNF-α stimulated by LPS, thereby reducing the damage caused to microglia by these inflammatory factors (Shin et al., 2006). In a study, a model of left common carotid artery occlusion stroke to investigate the protective effects of AME on cerebral ischemia/reperfusion injury in rats. The findings indicated that after ischemia/reperfusion injury, AME significantly reduced the neurological deficit scores, improved motor coordination ability and spontaneous activity, reduced the levels of TNF-α, IL-1β, and IL-6, inhibited the NF-κB signaling pathway, reduced caspase-3 to block cell apoptosis, increased the levels of TNF receptor-associated factor family member-associated NF-κB activator-binding kinase 1 and interferon β, reduced MDA levels, and increased GSH and CAT levels in the brain. The mechanism of AME may be mediated by high mobility group box protein B1 (HMGB1) through the toll-like receptor-4 (TLR4)/NF-κB signaling pathway (Saeedan et al., 2023). These experimental phenomena and mechanisms indicate that AME has a good protective effect on ischemic brain injury.

3.3 Effect of AME on neurological diseases

3.3.1 Preventing Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of β-amyloid (Aβ) peptides, neurofibrillary tangles formed by hyperphosphorylated tau proteins, abnormal oxidative stress damage, inflammatory responses, and neurotransmitter disorders in affected brain regions (Breijyeh and Karaman, 2020). It is commonly treated with acetylcholinesterase (AChE) inhibitors and N-methyl-D-aspartate receptor antagonists. Although these medications can alleviate clinical manifestations, they do not reverse cognitive impairment, and are often accompanied by common side effects, including gastrointestinal symptoms, confusion, dizziness, and headaches. (Chin et al., 2022).

Overproduction of Aβ leads to the formation of neurofibrillary plaques in the brain, which subsequently accumulate in the blood vessels to cause cerebral amyloid angiopathy (Han et al., 2022). Therefore, inhibiting the production of Aβ and promoting its clearance are crucial for improving AD. In a study, researchers used the fluorescent dye thiazine 1,3,5-tetracarboxylic acid to investigate the inhibitory effect of various flavonoid compounds on Aβ aggregation and the structure-activity relationship of promoting Aβ fibril disaggregation. The results indicated that AME could inhibit the formation of $A\beta_{1\text{-}42}$ fibrils, had a better

affinity for $A\beta_{1-42}$ fibrils than for $A\beta_{1-40}$ fibrils. The hydrophilic group of AME was the key group for its function. Furthermore, among flavonoid compounds with different structures, AME with four hydroxyl groups could most effectively inhibit Aβ fibrillation and promote the disaggregation of pre-formed Aβ fibrils (Choi et al., 2020). Another study showed that increasing the number of methoxy groups on the AME parent structure weakened the inhibitory activity of AME on $A\beta_{40}$, and the connection of single bonds also affected the inhibitory activity (Sirimangkalakitti et al., 2019). A study elucidated how AME inhibits the aggregation of $A\beta_{42}$ peptide at the atomic level. AME bound to aromatic residues in the N-terminal of $A\beta_{42}$ peptide, forming a stable π–π interaction, which destabilized the fibril structure. Subsequently, AME utilized its hydrogen bond donor/acceptor specificity to disrupt the hydrogen bond potential of the fibril peptide backbone, thereby disrupting the fibril structure and promoting Aβ fibril disaggregation (Windsor et al., 2021).

A study explained the anti-AD function of AME from the perspective of receptor-mediated endocytosis for Aβ clearance. AME can significantly increase Aβ uptake by mouse N2a neural cells through class A scavenger receptors, and then enter the lysosome within the cell, where it is degraded by relevant enzymes without causing cytotoxicity. The hydroxyl group in the molecular structure of AME enhances Aβ uptake, whereas the substitution of methyl groups decreases its uptake effect (Han et al., 2022). Another study identified AME from a library of polyphenolic compounds that can increase the activity and expression of neuropeptidase, an Aβ degradation enzyme, thereby delaying the progression of AD. Among them, the double-bond chemical structure in the C ring of the AME structure can significantly enhance neuropeptidase activity (Hori et al., 2023).

Research indicated that among various flavonoid compounds, AME exhibited the strongest neuroprotective effect, significantly inhibiting ROS-induced oxidative stress damage in SHSY5Y neuroblastoma cells. AME reduced DNA damage induced by etoposide DNA damage, whereas other flavonoid compounds increased cell death caused by DNA damage. Moreover, at a concentration of 2 μM, AME was most effective at reducing cytotoxicity induced by the $A\beta_{25-35}$ peptide in rat PC12 cells, indicating the therapeutic potential of AME in neurodegenerative diseases (Kang et al., 2005). Futher studies found that AME could differentially protect SH-SY5Y neural cells from various cytotoxic factors such as hydrogen peroxide, okadiac acid, and $A\beta_{25-35}$. Specifically, AME was able to inhibit okadiac acid-induced tau protein hyperphosphorylation, restore mitochondrial membrane potential to inhibit cell apoptosis, and protect microtubule structure and mitochondrial function. Furthermore, AME exhibited strong antioxidant capabilities against mitochondrial dysfunction and ROS-induced oxidative stress damage. AME also bound to β-secretase to inhibit its activity, thereby preventing the degradation of Aβ protein precursor to produce large amounts of Aβ (Zhang, 2014). In models of AD, AME could activate nuclear factor erythroid 2-related factor-2 (Nrf2) through affecting the adenosine monophosphate-activated protein kinase (AMPK)/GSK-3-β signaling pathway to exert antioxidant stress effects, ameliorating Aβ-induced neural function deficits and neuronal apoptosis (Chen et al., 2018). Although studies have shown that GSK-3-β is involved in the regulation of Nrf2 by AME, further verification is required to determine the mechanism by which AME affects GSK-3-β signaling-mediated tau phosphorylation. Tt was demonstrated through multiple experiments that AME, as a bifunctional chelator, can bind to various Aβ aggregates with high affinity and reduce their induced cytotoxicity. Additionally, AME can effectively chelate with Cu^{2+} and exhibit strong antioxidant activity, thereby preventing the formation of soluble Aβ oligomers and ROS-induced by metal ions in the brain (Sun et al., 2020).

Autophagy can degrade and recycle proteins produced by misfolding and damaged organelles, thereby maintaining protein homeostasis. The activation of autophagy in cells can effectively eliminate the accumulation of Aβ, thereby reducing the progression of AD (Zhang Z. et al., 2021). AME can improve the memory and cognitive impairment induced by $A\beta_{25-35}$ in the brains of mice and alleviate inflammation, oxidative stress, and the immune response in the hippocampus. Additionally, it can enhance autophagy by binding to multiple amino acid residues of the mTOR protein, thereby inhibiting further phosphorylation of mTOR and exerting a neuroprotective effect against AD (Cao et al., 2021). Experiments were conducted on neuronal cell damage induced by $A\beta_{1-42}$. It was found that AME can improve neuronal dysfunction induced by $A\beta_{1-42}$ in rats and inhibit NLRP3 inflammasome-induced pyroptosis by regulating AMPK/GSK-3-β signaling, thereby ameliorating the neurotoxicity caused by $A\beta_{1-42}$ in AD (Zhao et al., 2019).

Using the scopolamine-induced dementia mouse model, it was discovered that oral administration of AME can inhibit the activity of AChE and increase acetylcholine levels. Furthermore, it can improve the oxidative stress damage induced by scopolamine in mice, mainly by reducing MDA levels and increasing GSH activity, thereby ameliorating cognitive impairment (Ishola et al., 2013a). Addtionally, a study collected 50 flavonoid compounds with therapeutic potential for AD. Molecular docking experiments were conducted with the human α7 nicotinic acetylcholine receptor (α7nACHR) and found that after binding with AME, it exhibited the best affinity and good stability parameters (Singh et al., 2023). However, the effectiveness of AME as an α7nACHR agonist for treating AD requires further verification.

3.3.2 Treating Parkinson's disease

In Parkinson's disease (PD), melanin is released as neurons degenerate. Subsequently, it is recognized and ingested by microglia, leading to inflammatory damage. In patients with PD, the breakdown of dopaminergic neurons can be attributed to chronic neuroinflammation, which leads to the production and accumulation of Lewy bodies in the compact region of the substantia nigra in the brain. The main pathological features of PD are damage to dopaminergic neurons in the midbrain substantia nigra and the establishment of diffuse Lewy bodies, which subsequently lead to motor neuron dysfunction. Therefore, taking measures to inhibit neuroinflammation may be beneficial for alleviating PD clinical manifestations (Sharma et al., 2023).

Microglia, analogous to macrophages within the brain, can be activated by various stimuli to release a range of inflammatory mediators, thereby initiating an inflammatory response that may result in neural damage (Cai et al., 2022). AME has been shown to improve motor dysfunction and anxiety-like and depressive-like behaviors in the LPS-induced PD rat model. Mechanistic studies revealed that AME effectively inhibits the expression of pro-

inflammatory factors (TNF-α, IL-1β, IL-6, iNOS, COX-2) and enhances the expression of anti-inflammatory factors (IL-4, TGF-β, Arg-1, CD206), indicating that AME promotes the polarization of microglia towards the M2 anti-inflammatory phenotype. Furthermore, AME significantly ameliorates the reduction in tyrosine hydroxylase-positive neurons and the increase in α-synuclein expression observed in the model, suggesting that AME exerts neuroprotective effects in the LPS-induced PD model (Liu et al., 2024). It has been discovered that after LPS stimulation2 of rat astrocytoma cells (C6), AME significantly inhibited the production of nitrites, ROS, MDA, and TNF-α, upregulated GSH levels, and reduced intracellular oxidative stress (Ishola et al., 2013b). Activated ERK1/2 promotes cell death due to oxidative toxicity, but AME can alleviate oxidative stress damage caused by glutamate and ROS in HT22 neuronal cells by maintaining the activity of antioxidant enzymes and inhibiting ERK1/2 activity (Jeong et al., 2014). Molecular docking studies have revealed that AME exhibits strong binding affinity with the glutathione peroxidase 4 (GPX4) protein, which is involved in ferroptosis (Xiong et al., 2024). It was reported that AME can inhibit inflammation induced by ferroptosis in HT22 cells triggered by homocysteine. However, it is important to note that AME treatment results in decreased expression levels of both solute carrier family 7 member 11 (SLC7A11) and GPX4. Given that the SLC7A11/GPX4 signaling pathway requires the upregulation of these proteins to effectively suppress ferroptosis, the observed reduction in their expression following AME treatment may affect its efficacy in preventing ferroptosis (Wang et al., 2024).

In vivo and *in vitro* PD model studies demonstrated that AME can significantly reduce the expression of glial fibrillary acidic protein and lba1 markers in glial cells under inflammatory conditions, decrease the activation of caspase-3 and p21, and reduce the expression levels of IL-1β and iNOS and increase the Bcl-2/Bax ratio, through the PI3K/Akt and ERK signaling pathways. In this study, in a vitro mouse model induced by methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), AME was able to protect dopaminergic neurons and reduce striatal fiber loss (Cao et al., 2017). These findings suggest that AME can exert beneficial effects on dopaminergic neurons and glial cells, thereby ameliorating the clinical manifestations of PD. Furthermore, studies have shown a deregulated angiotensin-converting enzyme (ACE)/Ang II/angiotensin II receptor-1 (AT1R) axis is activated at the onset of PD, leading to free radical damage, cell apoptosis, and neuronal disruption. AME can bind to the mitochondrial assembly receptor (MASR) protein and activate the ACE2/Ang (1–7)/MASR, thereby neutralizing the neurodegenerative changes triggered by the ACE/Ang II/AT1R axis (Bhadauriya et al., 2023).

3.3.3 Treating epilepsy

The development of inflammation is closely related to the onset of epilepsy and its clinical progression. Brain inflammation can promote neuronal excitability, reducing the seizure threshold, thereby triggering seizures. Therefore, anti-inflammatory therapy can be used to prevent and treat seizures (Alsaegh et al., 2021). Studies found that AME improve pentetrazole-induced cognitive dysfunction by inhibiting the NLRP3 inflammasome, reducing the susceptibility to seizures and apoptosis in hippocampal neurons of mice. In this study, AME can also inhibit the mRNA expression of NLRP3, ASC, and caspase-1 in BV2 microglia induced by LPS, reducing the expression of inflammatory factors IL-1β, IL-18, and TNF-α. However, in the absence of inflammation, administration of AME to mice did not affect the expression of these proteins and inflammatory factors (Rong et al., 2019). Research indicated that AME can inhibit the activation and nuclear translocation of NF-κB p65 subunit in mice, thereby inhibiting the NF-κB signaling pathway to reduce the injury of hippocampal CA1 neural cells. AME can also reduce the production of inflammatory mediators NO and PGE-2, and inflammatory factors IL-6 and IL-β in neural cells. Furthermore, histological analysis found that AME can protect neurons after status epilepticus, inhibit the excessive discharge of hippocampal neurons, and shorten the duration of seizures (Zhang et al., 2015). These results indicate that AME has a good protective effect on hippocampal neuronal injury caused by epilepsy and has good anti-inflammatory effects. A study employed various computational methods and found that AME exhibits superior affinity for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and voltage-gated sodium ion channels (VGSC) receptors compared to phenytoin. Additionally, physicochemical and pharmacokinetic studies indicate that AME is suitable for oral administration, demonstrating favorable intestinal permeability and the ability to cross the blood-brain barrier, with no significant risk of toxicity (Salaria et al., 2024). These findings suggest that AME is a promising candidate for further research as a potential treatment for epilepsy.

4 Conclusion and future perspective

AME has been reported in multiple studies as the primary active pharmacological component from its plant sources, with a wide range of pharmacological effects (Yu et al., 2017; Li et al., 2021; Zhang et al., 2015). Due to the unique chemical structure of AME, its biological activity can be significantly influenced by multiple factors. Further studies could elucidate how various factors, such as photooxidation, affect the structural stability of AME. Additionally, pharmacokinetic studies have demonstrated that AME undergoes rapid metabolism in the body, resulting in low bioavailability. Therefore, modifying the natural structure or developing suitable drug formulations of AME is crucial for improving its therapeutic properties and enhancing its bioavailability.

The anti-inflammatory, antioxidant, and lipid-lowering effects of AME are notable, and recent studies have demonstrated its application in various cardiac and neurological diseases, suggesting that it may have significant anti-atherosclerotic effects. However, most of the current data on AME are derived from *in vitro* cell experiments, with limited *in vivo* animal testing. More comprehensive animal experiments are required to validate its pharmacological activity. One study has found that AME may have hepatotoxic and nephrotoxic effects (Li D et al., 2019). This warrants further investigation.

AME exhibits significant therapeutic effects on various cardiocerebrovascular diseases and neurological disorders, potentially functioning through multiple mechanisms. All these disease processes are accompanied by inflammatory responses, which are triggered by multiple factors. AME can affect multiple inflammatory mediators and mechanisms, suggesting that it has considerable potential for treating these diseases. However, the specific mechanisms of action involved in the effects of AME on inflammatory diseases require further research. Hemodynamic factors such as hypertension cannot be ignored in the

development of cardiocerebrovascular; however, the mechanisms and targets by which AME counters the effects of these factors remain unclear. Currently, there remains controversy regarding whether AME can exert a therapeutic effect on AD. Furthermore, the specific mechanisms by which AME influences the cellular uptake and clearance of Aβ also require further validation research.

In summary, AME exhibits multiple activities, indicating its potential as a natural drug for treating cardiocerebrovascular diseases and neurological disorders. Further studies on its pharmacokinetics and toxicology are required to ensure its safety and efficacy.

Author contributions

HZ: Writing–original draft. Y-MB: Writing–original draft. D-ML: Writing–original draft. GW: Writing–review and editing. JG: Funding acquisition, Writing–review and editing. LZ: Funding acquisition, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

AME	Amentoflavone
AGEs	Advanced glycation end-products
ASK1	Apoptosis signal regulating kinase 1
Akt	Protein kinase B
AD	Alzheimer's disease
Aβ	β-amyloid
AChE	Acetylcholinesterase
Ang	Angiotensin
ACE	Angiotensin-converting enzyme
AMPK	Adenosine monophosphate-activated protein kinase
AT1R	Angiotensin II receptor-1
Arg1	Arginase 1
AUC(0-t)	Area under the concentration-time cure
AUC(0-∞)	From time zero to all original drug elimination
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
α7nACHR	α7 nicotinic acetylcholine receptor
C_{max}	Maximum blood concentration
CL/F	Clearance
COX-2	Cyclooxygenase-2
CD36	Cluster of differentiation 36
C/EBP	Transcription factor CCAAT enhancer binding protein
CAT	Catalase
cGMP	Guanosine 3',5'-cyclic monophosphate
caspase	Cysteinyl aspartate specific protease
DPPH	1,1-diphenyl-2 trinitrophenylhydrazine
DOX	Adriamycin
ECs	Endothelial cells
ET-1	Endothelin-1
ERK	Extracellular signal-regulated kinase
Fasl	Fas ligand
F	Bioavailability
Fizz1	Found in inflammatory zone
GSK-3-β	Glucose synthesis kinase-3-β
GBE	*Ginkgo biloba* extract
GSH	Glutathione
GPX4	Glutathione peroxidase 4
HMGB1	High mobility group box protein B1
HSP-27	Heat shock protein 27
HFFD	High fructose and fat diet
HPLC	High-performance liquid chromatography
HUVECs	Human umbilical vein endothelial cells
hIAPP	Human islet amyloid polypeptide
IL	Interleukin
IκB	Inhibitor of NF-κB
iNOS	Nitric oxide synthase
LPS	Lipopolysaccharide
LDL	Low-density lipoprotein
$MRT_{0-\infty}$	Mean residence time
MRT_{0-t}	Average retention time for a certain period of time
MMPs	Matrix metalloproteinases
MCE	Mitotic clonal expansion phase
MDA	Malondialdehyde
MAPK	Mitogen-activated protein kinase
mTOR	Mechanistic target of rapamycin
MPTP	Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MASR	Mitochondrial assembly receptor
MIF	Macrophage migration inhibitory factor
NLRP3	NOD-like receptor thermal protein domain associated protein 3
NOX	NADPH oxidase
NF-κB	Nuclear factor-kappa B
NO	Nitric oxide
Nrf2	Nuclear factor erythroid 2-related factor-2
NRF-1	Nuclear respiratory factor-1
ox-LDL	Oxidized low-density lipoprotein
PGE-2	Prostaglandin E-2
PPAR	Peroxisome proliferator-activated receptor
PI3K	Phosphoinositide 3-kinase
PD	Parkinson's disease
PIGF-1	Placental growth factor-1
ROS	Reactive oxygen species
SOD	Superoxide dismutase
STING	Stimulator of interferon genes
SLC7A11	Solute carrier family 7 member 11
TG	Triglycerides
Trx	Thioredoxin
TrxR-1	Thioredoxin reductase-1
T_{max}	Time to peak concentration
$T_{1/2}$	Biological half-life
T2DM	Type 2 diabetes model
TNF	Tumor necrosis factor
TGF	Transforming growth factor
TLR4	Toll-like receptor-4
TFAM	Mitochondrial transcription factor
UGT1A	Uridine diphosphate glucuronosyltransferase 1 family, polypeptide A

V/F	Apparent distribution volume
VSMCs	Vascular smooth muscle cells
VEGF	Endothelial growth factor
VGSC	Voltage-gated sodium ion channels
Ym1	Chitinase-like protein 3

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