NEW TRENDS AND APPROACHES IN PERIOPERATIVE PHARMACOTHERAPY: AN UPDATE

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NEW TRENDS AND APPROACHES IN PERIOPERATIVE PHARMACOTHERAPY: AN UPDATE

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EDITORIAL
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Editorial: New Trends and Approaches in Perioperative Pharmacotherapy: An Update

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Keywords: perioperative pharmacotherapy, postoperative recovery, anesthesia, adverse drug effects, enhanced recovery after anaesthetic, postoperative nausea and vomiting

Editorial on the Research Topic

New Trends and Approaches in Perioperative Pharmacotherapy: An Update

The perioperative period poses significant additional risks on patients, who already suffer from various health-related problems. Even with advances in surgical treatment, anesthesia safety, diagnostic imaging, and intensive care, the perioperative period is still associated with serious morbidity and mortality. According to Bhatia et al. (2021), the 30-day mortality in patients undergoing non-cardiac surgery remains as high as 1.3–1.9%, and the incidence of postoperative myocardial infarction reaches 4.2–6.3%. Cerebrovascular and pulmonary complications, impaired glycemic control, metabolic derangement, infections, and iatrogenic complications all add up to the long list of potential risks and adverse effects encountered in the perioperative period (Ben-Shlomo and Melmed, 2003). Current approaches to perioperative patient care highlight the requirement for adequate pain control using multimodal therapy: opioids, non-opioid analgesics, gabapentinoids, pain modifiers, regional blocks, and other therapies. Post-surgical enhanced recovery protocols have been developed in recent years allowing for shortened recovery time and improved patient satisfaction.

Patients undergoing surgery commonly receive chronic treatment with anti-hypertensives, anti-arrhythmic drugs, anticoagulants, chemotherapeutic drugs, and other medications. Many of these drugs have the potential to cause serious drug-drug interactions and perioperative adverse effects (Pai et al., 2017; Pfeifer et al., 2021).

Undoubtedly, questions related with the perioperative patient care and drug management in this patient group are among the most discussed in medical literature.

The Society for Perioperative Assessment and Quality Improvement highlights the importance of evidence-based approach to perioperative care and medication management (Pfeifer et al., 2021).

Taking into account all these trends and advances in patient care, as well as advances in surgical care and complexity of surgical procedures, our editorial team decided to invite medical professionals to share with the readers their clinical experience and the results of their research in the constantly changing field of perioperative pharmacotherapy. Our decision was also based on success of our previously published topic dedicated to perioperative pharmacotherapy, which was viewed over 109,000 times.

The current update of our topic is an attempt to put together the recent advances in the field of perioperative pharmacotherapy and present opinions and results of health care professionals from different hospitals and countries.

The manuscripts that were included in the topic cover several important aspects of patient care. Questions discussed include drug interactions and adverse drug reactions during anesthesia, coagulation management in patients undergoing liver transplant surgery, interaction of cigarette smoking and drugs used during anesthesia, efficacy of antifibrinolytic

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therapy in major spinal surgery, use of vasopressors in free tissue transfer in head and neck surgery, and many other important problems.

The efficacy of prevention and treatment of postoperative nausea and vomiting (PONV) is an important component of perioperative care and one of the criteria to assess quality of care. It is well known that many drugs used in PONV management prolong the QTc on the EKG. However, there are insufficient data in literature related to PONV management in patients who present with an already prolonged QTc. A mini-review in our topic presents this important question attempting to focus the attention of clinicians and researchers on this important problem and encouraging further discussion and research to find an optimal strategy for PONV management in this patient category.

As a conclusion, surgery and anesthesiology are actively changing disciplines, and effective treatment of patients undergoing surgery is only possible with an up-to-date knowledge of the ongoing trends and advances in perioperative pharmacotherapy.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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OPINION
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Perioperative Management of Patients With Ankylosing Spondylitis Undergoing Spine Surgery

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Keywords: ankylosing spondylitis, spine surgery, spine deformity, perioperative management

Ankylosing spondylitis (AS) is the most frequent type of seronegative spondyloarthropathy, which mainly involves the axial spine. In advanced cases, the chronic inflammatory process can cause fibrosis and calcification, which leads to loss of flexibility and fusion of the vertebrae, resembling a "bamboo", with a fixed posture, generally known as chin-on-chest deformation.

The functional limitations of this particular deformity are significant: the patients are unable to look forward, make visual contact, and have difficulty eating. All these factors with typically concomitant osteoporosis will significantly increase the risk of spinal injury with severe instability, deformity, and, most importantly, deteriorating neurological function or paralysis requiring spinal surgery. Common indications for spinal intervention on patients with AS are:

- Uncontrolled, severe and continuous back and neck pain
- Deteriorating neurological deficit caused by spinal deformity
- Unstable spinal fracture
- Inability to hold the head up and see horizontally
- Serious difficulties in eating and drinking because of spinal deformity.

Spinal surgery on these patients is related to a greater risk of surgical and anesthesia-related complications—such as difficult intubation with necessity of awake fiberoptic intubation, infection, need for blood transfusions, respiratory and cardiac problems, and renal dysfunction postoperatively (Puvanesarajah et al., 2017). Therefore, assessment of medical comorbidities, associated with both the disease and the treatment of AS is an important component of the medical clearance before surgery.

Cardiovascular Concerns: According to the American Heart Association and American College of Cardiology (ACC/AHA) cardiac risk assessing guidelines in preoperative surgical preparation of patients with AS, cardiovascular disease is advanced if functional capacity is not reached to a minimum of four Metabolic Equivalents (METS) (Fleisher et al., 2014). These patients with poor functional capacity (<4 METS) are at greater cardiac risk (Goodman and Bass, 2018).

Pulmonary Concerns: Significant spinal deformity in AS can result in restrictive respiratory physiology, with notable decreases in vital lung capacity (Berdal et al., 2012). This may make ventilation during surgery challenging, especially when a prone position is anticipated. For those patients, aggressive pulmonary hygiene in the postoperative period can be encouraged

Neuromuscular Concerns: A thorough physical examination should be performed to document any preoperative sensory or motor deficits, especially in patients undergoing spinal surgery.

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Notation of any motor weakness is important as this may affect the plan for intraoperative neuromonitoring and the anesthesia (Sciubba et al., 2008).

Renal Concerns: Since AS patients receive non-steroidal anti-inflammatory drugs (NSAID) for a long period, interstitial nephritis may be present due to NSAID use. Advanced AS may be associated chronic kidney dysfunction due to amyloidosis, IgA-nephropathy, and tubulointerstitial nephritis (Ye et al., 2019).

Bone loss Concerns: Taking into consideration that osteoporosis is a common complication of AS, with an incidence between 18.7% and 62%, all patients undergoing spinal surgery should be treated before and after surgery for osteoporosis (van der Weijden et al., 2012).

According to the Assessment of SpondyloArthritis International Society/European League Against Rheumatism (ASAS-EULAR) management suggestions for AS, NSAIDs are considered the first-line drug treatment. To prevent further progression of the disease and achieve remission with improved quality of life, disease-modifying antirheumatic drugs (DMARD) are required: synthetic (such as sulfasalazine) and biological (anti-TNF-α agents such as infliximab, etanercept, adalimumab, golimumab, etc. as well as inhibitors of IL-17-secukinumab) (van der Heijde et al., 2017).

Taking into account the need for chronic therapy and associated side effects of those drugs, patients undergoing surgery need dosage adjustment.

NSAIDs: The perioperative use of NSAIDs or aspirin may be associated with bleeding complications. This is because NSAID-induced inhibition of COX-1 with reduction of thromboxane A2, which, in turn, will decrease vasoconstriction and platelet aggregation and increase the bleeding time. This effect is reversed only after drug withdrawal. Considering the average half-life of an NSAID, they must be withdrawn at some point prior to surgery. (Franco et al., 2017). On the other hand, in patients, whose cardiovascular risk exceeds the intraoperative benefit (for example, patients with cardiac stents), it's recommended to continue the aspirin during the perioperative period (Oscarsson et al., 2010; Gerstein et al., 2015). In such situations, the surgeon must be alerted and prepared for bleeding during surgery, even though cardiac doses of aspirin, in general, are not associated with a significant risk of bleeding (Vetter et al., 2014). Since COX-2 inhibitors have little effects on platelet function, there is no need to suspend them during the perioperative period. (Leese et al., 2000; Franco et al., 2017). However, this group of drugs is associated with the risk of significant cardiovascular adverse outcomes.

Glucocorticosteroids (GCS): Patients continuously taking GCS are at risk of intraoperative hemodynamic instability and postoperative infection. A few small randomized controlled trials and systematic reviews demonstrate that there are no significant hemodynamic changes between patients receiving a daily dose of GCS compared to those receiving "stress-dose steroids" preoperatively. (Goodman et al., 2017). Therefore it's recommended to use the usual daily dose of GCS or prednisone less than 20 mg/day, rather than the "stress dose" with regards to infection risk (Somayaji et al., 2013).

DMARDs: Non-biologic synthetic DMARDs are recommended to continue at the time of surgery since it was shown that the risk of infections is not increased. Perioperative continuation of DMARDS also decreases the risk of exacerbation of the main disease after the surgery (Goodman et al., 2017).

Systematic reviews and meta-analyses of biologic DMARDs revealed an increased risk of serious infections. Most commonly the respiratory and urinary tract infections, skin, and opportunistic infections are developed, including hepatitis B and C, tuberculosis, and various fungal infections such as histoplasmosis (Nard et al., 2015).

Therefore, all current biologic DMARDs should be withdrawn before the surgery and it is important to schedule the procedure after the active period of these drugs (Goodman et al., 2017).

In conclusion, proper perioperative management, consisting of preoperative patient planning, as well as intraoperative and postoperative patient monitoring and care, are required. Further investigations are needed to build up a reliable, evidence-based strategy of effective perioperative management of AS patients undergoing spine surgery.

AUTHOR CONTRIBUTIONS

AZ and KG contributed to the writing of the manuscript.

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REVIEW
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The Use of Vasopressor Agents in Free Tissue Transfer for Head and Neck Reconstruction: Current Trends and Review of the Literature

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Background/Objectives: Microvascular free tissue transfer has become essential to head and neck reconstruction and recent advancements in microvascular surgery have led to excellent surgical outcomes. However, there continues to be controversy and a stigma associated with the use of perioperative intravenous vasopressor agents among both surgeons and anesthesiologists. Due to concern for vasoconstriction of peripheral vasculature flowing to the denervated tissue flap, there remains concerns about potential thrombosis, decreased tissue perfusion and ultimately flap failure. This topic becomes even more important as vasopressors play an essential role in new Extended Recovery After Surgery (ERAS) protocols being put in place to optimize postoperative recovery for patients. The purpose of this study was to comprehensively review the role and safety as well as discuss current trends with intraoperative vasopressor agents in free tissue transfer for head and neck reconstruction.

Methods: A scoping literature review was conducted of all studies that examined the use of vasopressor agents during head and neck free flap tissue transfer. Primary and secondary outcomes included free flap survival, arterial thrombosis, venous congestion, need for revision surgery, and other postoperative complications.

Results: One prospective and nine retrospective studies were identified. Phenylephrine and ephedrine were the most common vasopressors reported; the rate of vasopressor use ranged from 53% to 85% and administration methods included both bolus and infusion. The included studies did not show any significant association between the use of vasopressors and free flap failure, pedicle thrombosis, or other flap complications.

Conclusion: The administration of vasopressors during microvascular free tissue transfer for head and neck reconstruction does not seem to be associated with increased flap failure rates or other postoperative morbidities. Moreover, vasopressors may provide overall improved hemodynamic stability and help to limit overall fluid administration and subsequent postoperative complications. Additional prospective investigation is

warranted to further elucidate and establish evidence-based recommendations regarding the type, timing, and dose of vasopressors to further enhance free flap survival and patient outcomes.

Keywords: vasopressors, anesthesia management, microvascular surgery, head and neck reconstruction, free tissue transfer

INTRODUCTION

Microvascular free tissue transfer (MFTT) has become an essential component of head and neck reconstruction (Monroe et al., 2010). While advancements have led to excellent surgical outcomes with MFTT success rates routinely exceeding 95% (Densky et al., 2019), there remains controversy regarding the use of intraoperative intravenous vasopressor agents among both surgeons and anesthesiologists during these procedures (Harris et al., 2012; Chan et al., 2013; Ibrahim et al., 2014; Swanson et al., 2016; Wax and Azzi, 2018). Due to intraoperative hypotension, it is commonplace for teams to have ongoing discussions during these cases to select intraoperative fluid administration or vasopressor therapy medication and dosing. Theoretically, vasoactive agents have been feared to cause vasoconstriction, thereby potentially increasing the risk of thrombosis and flap failure (Cordeiro et al., 1997; Harris et al., 2012; Ibrahim et al., 2014). This was supported, early on, by animal models suggesting that phenylephrine decreased flow through the flap pedicle in musculocutaneous island flaps (Cordeiro et al., 1997). Thus, vasopressors have often been historically and anecdotally associated with potential postoperative complications with 70% of surveyed surgeons prohibiting their use during microvascular surgery in a previous study (Motakef et al., 2015; Chang et al., 2017). More recently, however, studies have challenged this paradigm and demonstrated an increased prevalence of intraoperative vasopressor use in free flap reconstruction without a significant impact on MFTT outcomes (Monroe et al., 2011; Harris et al., 2012; Chan et al., 2013; Swanson et al., 2016; Fang et al., 2018; Goh et al., 2019).

While data and opinions regarding vasopressor use in MFTT are evolving, there is a lack of evidence-based guidelines that draw upon prospective studies. As a result, there exists a broad spectrum of practice regarding the administration and type of vasopressors used based on an institutional and personal basis. Moreover, this topic is especially relevant as vasopressor agents play an essential role in newly implemented Extended Recovery After Surgery (ERAS) protocols, which universally limit intraoperative fluid administration with goals to improve recovery time and outcomes. The purpose of this study is to comprehensively review the role, safety, and current trends of intraoperative vasopressor agents in MFTT for head and neck reconstruction.

METHODS

Literature Search Strategy

A comprehensive scoping literature review was conducted through the PubMed-NCBI, Google Scholar, and Scopus. The final search was completed in February 2020. The search encompassed terms "vasopressor" OR "vasoconstrictive agents" AND "microvascular free tissue transfer" OR "free flap" AND "head and neck reconstruction". An English-language filter was applied. Each database was searched from inception date until February 2020. The reference lists of all obtained articles were examined for additional studies meeting inclusion criteria. Two authors (AN and TF) independently conducted the searches. The senior author was consulted for inclusion of seminal articles on the topic (NS). The resulting studies were reviewed first through titles and abstracts followed by full manuscript review of abstracts meeting inclusion criteria upon initial review.

Exclusion Criteria and Outcomes

Studies not in English and those examining MFTT results outside of the head and neck were excluded. Additionally, case reports and small case series were excluded. For studies that included overlapping series of patients, the most recent study with the largest number of patients was selected. The primary variable examined was the type of vasopressor agent and secondary variables included method and timing of administration. The primary outcome was the rate of free flap failure. Secondary outcomes included arterial thrombosis, venous congestion, and other postoperative complications including revision surgery, intraoperative re-anastomosis, wound infections, dehiscence, or hematoma.

Review of Vasopressor Use in Head and Neck MFTT

The search returned 247 articles. After applying exclusion criteria and reviewing abstracts, eight studies (one prospective observational and seven retrospective) were identified for inclusion. The results of these studies are highlighted in **Table 1**.

Prevalence and Types of Vasopressors

The most commonly used vasoconstrictive agents in the selected studies were ephedrine and phenylephrine, used alone or in combination (**Table 1**). The rate of intraoperative vasopressor use in the selected studies ranged from 53.3% to 88.4% and administration methods included both intravenous bolus and continuous infusion (Monroe et al., 2010; Monroe et al., 2011; Harris et al., 2012; Chan et al., 2013; Rose et al., 2016; Chang et al., 2017; Fang et al., 2018; Farquhar et al., 2018).

Free Flap Failure Rates and Postoperative Complications

Across the included studies, flap failure rate ranged from 1.48% to 13.1% with no study identifying a statistically significant

TABLE 1 | Flap Outcomes and Postoperative Complications Among Studies Examining the Effect of Intraoperative Vasopressors in Head and Neck Reconstruction.

Reference	Type of study	Free flap (%)	Use of intraoperative vasopressor, n (%)	No use of intraoperative vasopressor, n (%)	Type of vasopressor	Flap failure	Postoperative complications
Fang et al. (2018)	R	NR	2637 (88.4%)	346 (11.6%)	CaCl (77%) Eph (45%) Phe (21%)[#]	No significant difference in free flap failure rates. VP: 39/2637 (1.48%) vs Non-VP: 6/346 (1.73%), p=0.715	Pedicle compromise: -Intra-operative: VP: 8/2637 (0.30%) vs non-VP: 1/346 (0.29%) p=0.965 -Postoperative: VP: 76/2637 (2.88%) vs non-VP: 16/346 (4.62%), p=0.081 Arterial compromise: VP: 38/2637 (1.44%) vs non-VP: 10/346 (2.89%), *p=0.048* Venous congestion: VP: 59/2637 (2.23%) vs non-VP: 12/346 (3.47%), p= 0.161
Farquhar et al. (2018)	R	ALT (25.7%) RF (23.5%) Fibula (24.1%) Pectoralis (4.12%) Scapular tip (10.6%) LD (2.94%) RA (6.47%) Serra A (1.17%) Scapula (0.59%)	84 (54.5%)	70 (45.5%)	NR	No significant difference in free flap failure rates. VP: 11/84 (13.1%) vs Non-VP: 7/70 (10.0%), p=0.537	Major complications:[$] VP: 37/84 (44.0%) vs non-VP: 30/70 (42.9%), p=0.882
Chang et al. (2017)	R	ALT (46.6%) RF (22.1%)) Fibula (15.3%)) VL (2.7%) LD (2.7%)	278 (66.3%)	141 (33.7%)	Eph (44.5%) Phe (48.4%) Eph + Phe (33.3%)	Total flap loss: 12/419 (2.86%) *No direct comparison between VP and no VP failure rate	Arterial complication, VP: 22/278 (7.9%) vs non-VP: 2/141 (1.42%), *p = 0.002* OR = 6 (1.26-28.4), *p = 0.024*
Rose et al. (2016)	R	RF (70.7%) Fibula (17.1%) ALT (4.07%) Jejunum (4.07%) RA (2.44%) DCIA (1.63%)	93 (75.6%)	30 (24.4%)	NE (49.5%) MTM (44.1%) NE + MTM (6.45%)	VP: 3/93 (3.23%) Non-VP: 1/30 (3.33%)	Cases requiring salvage procedure to sustain viability: VP: 8/93 (6.50%) non-VP: 3/30 (10.0%)
Chan et al. (2013)	R	Jejunum (100%)	81 (73.6%)	29 (26.4%)	Eph (42.7%) Phe (14.5%) Eph + Phe (42.8%)	2/2 AT: VP: 1/81 (1.23%) vs Non-VP: 2/29 (6.9%), p = 0.08 2/2 VT: VP: 1/81 (1.23%) vs Non-VP: 2/29 (6.9%), p = 0.08	Late stricture formation: VP: 2/81 (2.47%) vs non-VP: 1/29 (3.4%), p = 0.12 Intra-op anastomosis revision due to AT: VP 1/81 (1.23%) vs non-VP, 2/29 (6.9%), p = 0.08
Harris et al. (2012)	R	RF (32.3%) ALT (28.3%) Scapula (13.9%) Fibula (12.3%) LD (6.2%)	320 (66%)	165 (34%)	Phe (34.7%%) Eph (25.6%) Phe + Eph (37.2%) Other (2.50%)	No significant difference in free flap failure rates. VP: 8/320 (2.5%) vs Non-VP: 3/165(1.81%), p = 0.76	VP: 18/320 (5.6%) vs vs non-VP: 8/165 (4.8%), p = 0.72[!] Timing of intra-op VP administration not significantly associated with adverse flap outcomes (p = 0.39)

(Continued)

TABLE 1 | Continued

Reference	Type of study	Free flap (%)	Use of intraoperative vasopressor, n (%)	No use of intraoperative vasopressor, n (%)	Type of vasopressor	Flap failure	Postoperative complications
Monroe et al. (2011)	P	RF (43.2%) ALT (16.6%) Fibula (15.4%) LD (10.1%) RA (8.28%) Jejunum (3.55%) Ulnar (1.77%) Scapula (1.18%)	90 (53.3%)	79 (46.7%)	Phe (63.0%) Eph (52.0%)	VP: 4/90 (4.44%) vs Non-VP: 2/79 (2.53%) 90% CI: -1.4 to 5.2	Total flap complications: VP: 34/90 (37.8%) vs non-VP: 34/79 (43.0%), p = 0.48 Medical complications: VP: 5/90 (5.56%) vs non-VP: 9/79 (11.4%), p= 0.17 Hematoma: VP: 8/90 (8.89%) vs Non-VP: 7/79 (8.86%), p = 0.99 Wound dehiscence: VP: 9/90 (10.0%) vs Non-VP: 9/79 (11.4%), p = 0.77 Infection: VP: 6/90 (6.67%) vs Non-VP: 6/79 (7.59%), p = 0.81 Anastomotic revision: VP: 3/90 (3.33%) vs non-VP: 2/79 (2.53%), p = 0.76 Other unspecified complications: VP: 7/90 (7.78%) vs Non-VP: 5/79 (6.33%), p= 0.76
Monroe et al. (2010)	R	RF (38.5%) RF w/bone (7.10%) Fibula (13.6%) ALT (12.4%) RA (10.1%) LD (7.10%) Ulnar (4.14%) Scapula (4.14%) Jejunum (2.96%)	139 (86.9%)	30 (13.1%)	Phe (33.0%) Eph (15.0%) Phe + Eph (51.0%) Dopa (1.00%)	No significant difference in free flap failure rates. VP: 4/139 (2.9%) vs Non-VP: 2/30 (6.7%), p= 0.29	Total flap complications: VP: 40/139 (28.7%) vs non-VP: 9/30 (30.0%), p = 1.00 Infection: VP: 9/139 (6.47%) vs non-VP: 1/30 (3.33%), p = 1.00 Dehiscence of fistula: VP: 10/139 (7.19%) vs non-VP: 2/30 (6.67%), p = 1.00 Hematoma: VP: 9/139 (6.45%) vs non-VP: 2/30 (6.67%), p = 1.00 Partial Loss: VP: 5/139 (3.6%) vs non-VP: 1/30 (3.33%), p = 1.00 Pedicle thrombosis: VP: 11/139 (7.91%) vs non-VP: 5/30 (1.67%), p = 0.17

R, retrospective study; NR - not recorded; CaCl, calcium chloride; Eph, ephedrine; Phe, Phenylephrine; VP, vasopressor; ALT, anterolateral thigh; RF, radial forearm; LD, latissimus dorsi; RA, rectus abdominis; Serr A, serratus anterior; VL, vastus lateralis; HR, adjusted Hazard ratio for logistic regression; DCIA, deep circumflex iliac artery; NE, norepinephrine; MTM, metaraminol; AT, arterial thrombosis; 2/2, secondary to; P -prospective observational; CI, confidence interval; Dopa, Dopamine.

#- Did not specify type of vasopressor used for head and neck reconstructions.

$- included reoperation, fistula, myocardial infarction, emergent tracheostomy, flap death, serious infection, and pulmonary embolus in the 30-day postoperative period.

! - complications not specified.

difference between vasopressor and non-vasopressor groups. In the largest study, Fang et al. identified 2,983 patients who underwent head and neck MFTT and found that intraoperative vasopressor use was not associated with an increase in free flap failure rates (1.48% vs 1.73%, p = 0.72) (Fang et al., 2018).

Alternatively, in a study of 110 free jejunal flaps, Chan et al. observed no significant relationship between the use of intraoperative vasopressors and the need for intraoperative re-anastomosis, free flap failure rate, or long-term stricture rate (Chan et al., 2013). Similarly, Harris et al. also showed no statistically significant difference between the vasopressor and non-vasopressor group with regards to complete flap failure (2.5% vs 1.8%, p = 0.76) or major flap complication rate, defined as the total proportion of flaps that failed or required revision surgery (5.6% vs 4.8%, p = 0.72) (Harris et al., 2012).

In contrast, Chang et al. observed that the use of vasopressors was an independent risk factor for arterial complications (odds ratio (OR) = 6; p = 0.02) (Chang et al., 2017). Despite a three-fold higher risk for emergent return to the OR and additional major surgical complications, patients with intraoperative arterial complications did not have higher rates of free flap failure. Thus, it appears most free flaps were salvaged in this study when arterial issues arose. Unfortunately, there was no direct comparison for free flap failure rates between the vasopressor group and non-vasopressor group (Chang et al., 2017).

Timing of Administration and Dosage

Chan et al. also demonstrated no relationship between the timing of vasopressor administration (i.e. prior to, during, or after free flap harvesting) and postoperative failure rates (Chan et al., 2013). An additional study concluded that pedicle compromise and flap failure rates were not associated with timing of intraoperative vasopressor use (p = 0.106 and p = 0.162, respectively) (Fang et al., 2018). Harris et al. showed no significant association between adverse flap outcomes and the timing of intraoperative vasopressors (first 3 h of the case, middle of the case, and last 3 h of the case; p = 0.39) or the cumulative dosage with either phenylephrine or ephedrine (p = 0.43 and p = 0.37, respectively) (Harris et al., 2012). In a prospective observational study, Monroe et al. observed no association between the total vasopressor dose administered and flap failure rates (Monroe et al., 2011).

DISCUSSION

The use of intraoperative vasopressors has long been debated among microvascular surgeons due to concerns for free flap failure and associated postoperative complications. Due to the lack of prospective data and heterogeneity of previous studies that include various defect sites and reconstruction methods, there is a lack of consensus on the use of intraoperative vasopressors.

Theoretical Risk of Vasopressors

This controversy likely stems from the conventional theory that vasoactive drugs may result in vasospasm, decreased flap perfusion, and subsequent flap failure in a denervated flap (Godden et al., 2000; Harris et al., 2012; Goh et al., 2019). Of note, these postulations are primarily based on experimental animal models with conflicting results (**Table 2**) (Banic et al., 1997; Cordeiro et al., 1997; Massey and Surgery, 2007; Lecoq et al., 2008; Scholz et al., 2009; Eley et al., 2013).

During free flap harvest, it has been postulated that local catecholamines are released due to the activation of sympathetic fibers induced during tissue dissection (Banbury et al., 1999). Once the local supply of catecholamines is depleted, the acute hyperadrenergic phase is followed by a non-adrenergic phase with possible increased collateral blood flow, and then by an increased adrenergic phase due to loss of modulating autonomic input (Banbury et al., 1999; Godden et al., 2000; Lecoq et al., 2008; Raittinen et al., 2016). The exact onset of adrenergic hypersensitivity that occurs with sympathetic denervation is unclear but seems to occur in a delayed fashion ranging from 48 h to 2 weeks (Banbury et al., 1999). Regardless, it appears that denervated soft-tissue does not respond in the same manner as the rest of the body when exposed to vasopressors. In animal studies, Lecoq et al. and Cordeiro et al. both demonstrated increased microcirculation to flap tissue (cutaneous and musculocutaneous pedicled flaps, respectively) secondary to increased mean arterial pressure (MAP), while there was decreased flow in normal tissue (Cordeiro et al., 1997; Lecoq et al., 2008). Moreover, in the only animal study to specifically examine the effects of flap perfusion in a free flap model, Banic et al., demonstrated no adverse effects on pedicle blood flow or free flap microcirculation with the systemic use of phenylephrine (Banic et al., 1997). Thus, the use of vasopressors intraoperatively may actually increase flap perfusion due to improved overall MAP without significant deleterious effects from sympathectomy (Rizzoni et al., 2000; Goh et al., 2019).

Physiology of the Autonomic Nervous System and Vasopressors

The autonomic nervous system, divided into parasympathetic and sympathetic components, regulates nearly every bodily function associated with homeostasis. Of particular interest to the microvascular surgeon are the adrenergic α- and β-receptors, which affect vascular tone and cardiovascular function. The role of α-1 agonists is to promote smooth muscle contraction, resulting in increased systemic vascular resistance and mean arterial pressure (MAP). α-2 agonists counteract this effect by causing smooth muscle relaxation. Of note, α-2 agonists also contribute to platelet aggregation through activation of α-2 receptors on platelets (Hoffman et al., 1982). β-1 agonists primarily enhance cardiac output due to positive inotropic and chronotropic effects, while β-2 agonists cause smooth muscle relaxation in the lungs (Miller and Pardo, 2011). Perioperatively, a host of endogenous and synthetic adrenergic agonists are available for use, each with varying degrees of effect on adrenergic receptors and associated physiologic and side effects (**Tables 2** and **3**) (Manaker and Parsons; Barrett et al., 2007; Miller and Pardo, 2011; Goh et al., 2019).

A variety of vasoconstrictive agents have been studied in animal models and used in human free tissue transfers, but the

TABLE 2 | Summary of Commonly Used Vasopressors in Microvascular Surgery.

Vasopressor	Manuscript including agent	Description of agent	Pharmacologic target	Physiologic effects	Potential adverse effects	Flap effects based on experimental animal and human studies
Phenylephrine (Neo-Synephrine)	Chan et al. Chang et al. Fang et al. Harris et al. Monroe et al.	Synthetic non-catecholamine	Strong α-1 adrenergic agonist	Vasoconstriction (venous constriction stronger than arterial constriction) and increased systemic vascular resistance (SVR) Mean arterial pressure (MAP) augmented by increased SVR; minimal cardiac inotropic or chronotropic effects	Reflex bradycardia, hypertension, arrhythmias, decreased cardiac output (CO), visceral ischemia, extravasation necrosis	No change in flap blood flow with systemic administration (Banic et al., 1997) Decreased flap blood flow (Cordeiro et al., 1997) Decreased flap blood flow (Massey and Surgery, 2007)
Ephedrine	Chan et al. Chang et al. Fang et al. Harris et al. Monroe et al.	Synthetic sympathomimetic amine	Strong β-1 and α-1 adrenergic agonist Moderate β-2 agonist	Vasoconstriction 2/2 increased endogenous norepinephrine at post-synaptic receptors Increased inotropic/chronotropic effects due β activity Increased SVR due to α-1 receptor activity	Tachycardia, arrhythmias, splanchnic vasoconstriction	NA
Calcium chloride	Fang et al.	Inotropic and vasoactive agent	Ca/calmodulin-dependent kinase II (CaMKII) pathway	Increased calcium concentrations activate CaMKII pathway resulting in increased CO 2/2 ionotropic effects	Hypercalcemia, hypotension, arrhythmia, bradycardia, syncope	NA
Norepinephrine (Levophed)	Rose et al.	Synthetic and endogenous catecholamine	Strong α-1 adrenergic agonist Moderate β-1 agonist	Vasoconstriction (increased SVR) resulting in CO	Severe reflex bradycardia, visceral ischemia, hypertension	Increased blood flow (Eley et al., 2013)
Metaraminol	Rose et al.	Synthetic sympathomimetic amine	Strong α-1 agonist	Vasoconstriction (increased SVR) resulting in CO; indirectly releases endogenous norepinephrine	Bradycardia, hypertension, arrhythmia	NA
Dopamine (Intropin)	Monroe et al. (Monroe et al., 2010)	Synthetic and endogenous catecholamine	Moderate D1 agonist Dose dependent β-1 and α-1 agonist	Low dose - selective vasodilation (decreased SVR) Intermediate dose - increased stroke volume and CO primarily due to beta adrenergic activity High dose - vasoconstriction (increased SVR and CO) due to alpha activity	Tachycardia, hypotension, arrhythmias, polyuria, extravasation necrosis	No change in flap blood flow (Cordeiro et al., 1997)
Dobutamine (Dobutrex)	NA	Inotropic agent	Strong β-1 adrenergic agonist Moderate β-2 agonist	Vasodilation (decreased SVR) Increased CO due to inotropic and chronotropic effects	Tachycardia, arrhythmia, headache, nausea	Increased flap flow (Cordeiro et al., 1997) Increased flap flow (Eley et al., 2013) Increased pedicle blood flow (Scholz et al., 2009)
Epinephrine (Adrenalin)	NA	Synthetic and endogenous catecholamine	Strong β-1 and α-1 adrenergic agonist Moderate β-2 agonist	Low dose - increased CO due to inotropic/chronotropic effects (β activity) High dose -Increased SVR and CO due to α-1 receptor activity	Tachycardia, arrhythmias, angina, extravasation necrosis, splanchnic vasoconstriction, pulmonary edema	Increased flap flow (Massey and Surgery, 2007) Decreased flap flow (Eley et al., 2013)
Vasopressin	NA	Endogenous hormone *vasopressor-like effects occur during sepsis/shock	Specific vascular (V-1) and renal (V-2) receptors	Vasoconstriction due to increased intracellular calcium; increased MAP Decreases nitric oxide-mediated vasodilation	Myocardial ischemia, arrhythmias, hyponatremia, bronchospasm, skin necrosis	NA

TABLE 3 | Physiologic Effects of Common Vasopressors.

Drug	Common dosing range (μg/kg/min)	α-1	β-1	β-2	Vasopressin-1
Dobutamine	2–20	+	+++	++	0
Dopamine	2–20	++	+++	++	0
Epinephrine	0.01–0.15	+++	++	+	0
Norepinephrine	0.01–0.1	+++	++	++	0
Phenylephrine	10–20	+++	0	0	0
Vasopressin	0.01–0.07	0	0	0	+++
Drug	**Common dosing range (μg/kg/min)**	**Mean Arterial Pressure**	**Heart Rate**	**Cardiac Output**	**Systemic Vascular Resistance**
Dobutamine	2–20	+	+	+++	–
Dopamine	2–20	+	+	+++	+
Epinephrine	0.01–0.15	+	++	++	++
Norepinephrine	0.01–0.1	+++	–	–	+++
Phenylephrine	10–20	+++	0	0	+++

most commonly used vasopressors in the selected head and neck studies were ephedrine and phenylephrine. The prevalence rates in head and neck reconstruction was fairly common and range between 53.3% and 88.4%. Vasopressors are often required for patients undergoing general anesthesia in order to maintain MAP as volatile anesthetic agents can decrease SVR as well as blood loss, hypothermia, and insensible fluid losses. Phenylephrine is a commonly used α-1 adrenergic agonist with mixed results in animal models regarding flap perfusion (Banic et al., 1997; Cordeiro et al., 1997; Massey and Surgery, 2007). Ephedrine is an indirect sympathetic agonist with primarily strong β-1 and β-2 effects and weak α-1 stimulation. It is considered to be a suitable option in free tissue transfer due to lesser effects on peripheral vasoconstriction.

Vasopressor Use in MFTT

While conflicting data exists, the majority of included studies indicate no increase rate of free flap failure with various vasopressor use (**Table 1**). Although, Chang et al. showed that the use of any vasopressor was a significant risk factor for arterial complication (Chang et al., 2017). However, this finding may be confounded by the underlying etiology of hypotension that resulted in the need for vasopressor use (Chang et al., 2017). It is also important to note that in this study arterial compromise did not significantly affect overall flap survival.

It has been postulated that a perforator flap may be more susceptible to intraoperative and postoperative complications with the use of vasopressors due to smaller caliber vessels. However, Harris et al. observed no significant association between the use of intraoperative vasopressors and postoperative complications specifically in free perforator flaps ($p = 0.22$) (Harris et al., 2012). In a prospective study of 24 patients undergoing head and neck resection and free flap reconstruction, Eley et al. observed that postoperative use of dobutamine and norepinephrine improved free flap skin blood flow (Eley et al., 2013). This increase in free flap perfusion with dobutamine has been observed by others and may be due to the β-adrenergic selectivity and isolated inotropic characteristics (Cordeiro et al., 1997; Suominen et al., 2004; Scholz et al., 2009). In a randomized controlled trial, Raittenen et al. randomized 25 patients undergoing radial forearm free flaps into three groups: dopamine, norepinephrine, and a control group with the goal vasopressor administration to maintain a MAP of 80 to 90 mmHg. The continuous partial pressure of oxygen and lactate to pyruvate ratio was monitored intraoperatively and for 72 h postoperatively *via* a subcutaneous catheter in the free flap. The authors found no difference in free flap failure rate, complication rate, or in the aforementioned clinical variables among the three groups (Raittinen et al., 2016). While small, this study represents the highest level of evidence available when interrogating the use of vasopressors and concludes that these agents can be safely used in MFTT.

Moreover, in a recent meta-analysis, Goh et al. observed no significant difference in total flap failure rate between vasopressor (71/3444) and non-vasopressor (25/349) groups who underwent head and neck reconstruction (2.1% vs 3.3%; OR = 0.91, p = 0.72) (Goh et al., 2019). A separate meta-analysis by Swanson et al. demonstrated similar findings and showed that intraoperative vasopressors had no effect on the incidence of flap failure (2.9% vs 3.6%; OR = 0.68, p = 0.48) or complication rates (16.8% vs 18.6%; OR = 0.92, p = 0.71) (Swanson et al., 2016).

Intravenous Fluid and ERAS Implications

The regulation of regional blood flow and overall hemodynamics including intraoperative MAP is a critical factor to maintain microvascular flap perfusion and decrease postoperative complications and morbidity (Sigurdsson, 1995; Haughey et al., 2001; Ibrahim et al., 2014; Goh et al., 2019). Many patients undergoing head and neck reconstruction are at risk for intraoperative hypotension due to anesthetic agents, opioid analgesics, prolonged operative times, blood loss, insensible fluid loss, hypothermia, and other associated medical co-morbidities (Sigurdsson, 1995; Chan et al., 2013; Raittinen et al., 2016; Goh et al., 2019). The administration of intravenous fluids (IVF) is often the first line treatment for acute hypotension prior to the use of vasopressors. The use of IVF may combat acute changes in blood pressure, but excessive IVF administration during prolonged surgical cases can result in flap edema, decreased flap microcirculation, and flap complications (Haughey et al., 2001; Goh et al., 2019). Free flaps are likely predisposed to significant edema due to the absence of lymphatic drainage pathways and poor interstitial fluid reabsorption secondary to flap denervation (Sigurdsson, 1995; Goh et al., 2019). Moreover, excessive fluid

resuscitation can lead to pulmonary edema and associated cardiopulmonary complications, which can also lead to subsequent deleterious flap and patient outcomes (Fang et al., 2018). Hand et al. observed that patients suffering perioperative complications received on average 525 mL more crystalloid than patients without complications (Hand et al., 2015). Similarly, Eskander et al. noted a 1.21 fold increased risk of wound-healing complications with each additional liter of crystalloid administered intraoperatively (Eskander et al., 2018). On the contrary, under-resuscitation can also result in increased free flap complications due to poor flap perfusion, worsened by further hypotension secondary to anesthetic agents (Sigurdsson, 1995). Thus, when faced with cardiovascular instability intraoperatively, anesthesiologists and microvascular surgeons must balance the use of vasoactive medications and fluid administration in an effort to improve free flap and patient outcomes.

Free tissue transfer for head and neck reconstruction often requires prolonged operative times and long hospitalizations (Won et al., 2019). ERAS protocols specific to head and neck surgery have been proposed and implemented to help reduce surgical complications and enhance recovery by utilizing a multimodal and multidisciplinary approach (Coyle et al., 2015; Dort et al., 2017). Goal-directed fluid replacement (equal weight in the immediate pre- and postoperative period) is one of several ERAS principles (Dort et al., 2017). The recent addition of stroke-volume variation to guide IVF administration has been shown to reduce length of stay and medical complications in head and neck patients (Abdel-Galil et al., 2010). Surgical teams must be vigilant in monitoring total IVF replacement in the postoperative period while also accounting for the volume of intraoperative fluid resuscitation in order to reduce flap edema, complications, and other perioperative morbidities. Thus, the use of intraoperative vasopressors to regulate systemic perfusion pressure may actually be a beneficial alternative to fluid administration to improve flap perfusion (Wax and Azzi, 2018).

Challenges With Assessing Vasopressor Use

As discussed, much of the literature available assessing vasopressor use has been limited to animal studies, other anatomic sites, or retrospective reviews. The lack of quality prospective data to draw from is a challenging issue. Currently, free flap success rates remain very high across the world, exceeding 95% at high volume centers (Densky et al., 2019). As such, many studies are under-powered and identifying statistically significant variables is not possible without extremely large patient volumes from multiple centers or across a time frame that introduces significant variations in clinical protocol. This type of data collection would certainly open the window for confounding variables and errors in data collection and analysis. Thus, there is no reasonable manner in which to perform a randomized, prospective trial in a safe, financially feasible, and ethical fashion. Lastly, patients undergoing surgery for advanced head and neck cancer frequently have significant medical comorbidities, which certainly play a role in the need for pressor support during general anesthesia. These comorbidities are difficult to control for in a meta-analysis due to inadequate powering.

Clinical Experience With Vasopressor Use

Anecdotally, we have seen success with the use of vasopressor agents intraoperatively at our institution as we have recently instituted improved ERAS protocols. Combined with the use of neuromuscular blockage to reduce systemic anesthesia requirements, a low-dose pressor drip throughout the case can help stabilize MAP and has been subjectively improved flap perfusion after anastomosis. Our anesthesiologists prefer the use of a low-dose phenylephrine drip when necessary to counter anesthesia-related reduction of SVR. In our experience, when we encounter issues related to flap perfusion with the use of vasopressor agents, it is related to bolus administration and typically occurs immediately prior to or just after flap re-perfusion. It is possible that wide fluctuations in MAP related to bolus administration of vasopressors are detrimental to the peripheral blood flow needed to perfuse the MFTT. We fear that bolus-style administration is most worrisome when performed around the time of flap re-perfusion as vascular compromise at this critical juncture can have long-lasting negative effects for the MFTT outcome. For this reason, we prefer a continuously-infused, low-dose agent, such as phenylephrine, when vasopressor administration is required. Phenylephrine remains the first-choice agent in these common settings at our institution.

Along with this approach, we stress the importance of clear communication with the anesthesia team to coordinate vasopressor timing, dosage, and agent. Of note, although a survey in 2015 showed that 70% of microsurgeons do not permit the use of vasopressors in non-emergent settings (Motakef et al., 2015; Chang et al., 2017), the retrospective studies reviewed here showed vasopressor use in 53.3% to 88.4% of surgeries (Monroe et al., 2010; Monroe et al., 2011; Harris et al., 2012; Chan et al., 2013; Rose et al., 2016; Chang et al., 2017; Fang et al., 2018; Farquhar et al., 2018). This high prevalence rate suggests that vasopressor agents are administered more frequently than surgeons are aware of, further emphasizing the importance of adequate communication.

As the majority of results highlighted in **Table 1** suggest, the use of intraoperative vasopressors is not associated with worse free flap outcomes. This is seen regardless of agent choice and administration timing during the case. However, a thoughtful methodology and communication between teams must be implemented. The judicious and appropriate use of intraoperative vasoactive agents can even be beneficial when utilizing ERAS protocols which are becoming commonplace at high volume centers. Postoperative free flap complications including arterial complications are often multifactorial in nature and can be confounded by associated medical co-morbidities making quality evaluation of these issues difficult (Chang et al., 2017).

CONCLUSION

Based on the results of the highlighted studies and recent meta-analyses, the use of intraoperative vasopressors appears to be safe in free tissue transfer for head and neck reconstruction. It is imperative to maintain open communication between microvascular surgeons and anesthesiologists in order to maintain a balance between the use of IVFs and vasopressors in the perioperative period. Prospective studies are warranted, taking advantage of ERAS protocols in place, to further examine the safety of intraoperative vasopressors on postoperative free flap outcomes and to establish evidence-based guidelines regarding the ideal type, dose, and timing of intraoperative vasopressor.

AUTHOR CONTRIBUTIONS

AN and NS contributed to the design and implementation of the project. AN, TF, ML, and NS performed the primary the literature review and data analysis. AN, ML, and NS wrote the manuscript after discussion, contributions, and edits from all authors (including AT, AA, EO, SK, MO).

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CASE REPORT
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Closure Device-Related Thrombosis After Anticoagulation With Dabigatran in Patients Undergoing Percutaneous Left Atrial Appendage Closure: Case Reports and Literature Review

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Percutaneous left atrial appendage closure (LAAC) is an effective and safe operation strategy for stroke prevention in patients who are diagnosed with atrial fibrillation (AF) but cannot tolerate long term anticoagulation medication. We presented four rare cases of thrombosis formation on the occluder device. After the LAAC operation was successfully performed on patients, they followed a course of anticoagulation with dabigatran (110 mg b.i.d.), and device-related thrombosis (DRT) occurred as indicated by a transesophageal echocardiogram (TEE) during the follow-up period. Regressions were achieved after replacing dabigatran with rivaroxaban or warfarin for more than 1 month. No thrombosis or bleeding-related complications occurred in subsequent follow-ups.

Keywords: atrial fibrillation, left atrial appendage closure, dabigatran, device-related thrombosis, novel oral anticoagulation

INTRODUCTION

Recently, percutaneous left atrial appendage closure (LAAC) has been developed as an effective and safe operation strategy for stroke prevention in patients who are diagnosed with atrial fibrillation (AF) but cannot tolerate long term anticoagulation (Glikson et al., 2019; Holmes et al., 2019; Skurk and Landmesser, 2020). The left atrium plays an important role in the formation of thrombosis for patients with AF, and about 90% of identified cases of left atrium thrombosis are located in the left atrial appendage (LAA) (Kirchhof et al., 2016; Saw et al., 2019). LAAC raises an intriguing concept mainly due to the combination of the reduction in thromboembolism and bleeding risks based on its technical success without the need for long-term pharmacological treatment (Hobohm et al., 2019; Masjuan et al., 2019; Wintgens et al., 2019).

Similar to other implanted devices in the human body, there is a requisite time for full endothelialization on occluders that are exposed to circulating blood (Cornelissen and Vogt, 2019). However, there was significant interindividual variability on anticoagulation before complete

endothelialization on devices, which might add to uncertainty on the duration of antithrombotic therapy during this vulnerable time for device-related thrombosis (DRT) (Massarenti and Yilmaz, 2012; Tang et al., 2017). Previous research reported that the incidence of DRT with oral anticoagulants (OAC) was lower than that with antiplatelet therapy (3.1 vs. 1.4%; p = 0.018) (Bergmann et al., 2017). However, the optimal anticoagulation regimen is uncertain owing to the lack of comparative clinical studies on different antithrombotic agents (mainly dabigatran and rivaroxaban). Previous studies have provided definitive evidence on the safety and efficacy of rivaroxaban for post anticoagulation of LAAC (Panikker et al., 2016), but little is known about the anticoagulation effects of dabigatran on LAAC. Current evidence shows that medication with dabigatran fails to prevent thromboembolic complications for patients after stents were implanted and mechanical heart valves replaced (Jaffer et al., 2015).

Given the recent increase in the prescription of novel oral anticoagulants (NOAC), such as dabigatran, for post-implantation anticoagulation after LAAC operations, it seems important to obtain a better understanding of the pharmacology and adverse effects during anticoagulation. Here, we report on four cases of occluder-related thrombosis anticoagulated with dabigatran in patients undergoing LAAC operations.

CASE STUDIES

Figure 1 presents progression of clinical picture in our patients.

Case 1

A 78-year-old woman (weight, 73 kg; height, 158 cm; BMI, 29.2 kg/m^2) developed paroxysmal AF with clinical presentation as palpitations after exercise accompanied by chest discomfort. The electrocardiogram (ECG) showed the typical pattern of AF: irregular RR intervals and no discernible, distinct P waves. She presented a history of hypertension for 10 years mediated with valsartan and amlodipine to control blood pressure. As her CHA_2DS_2-VASc score was 4 (female, elderly, and hypertensive), she now had a clear reason for anticoagulation and was temporarily treated with a vitamin K antagonist (VKA) within the therapeutic range [international normalized ratio (INR), 2.0–3.0). One year after warfarin initiation, she was referred to a tertiary cardiology center for further analysis of AF and was considered for the LAAC operation due to her unsuitability for continuous long-term OAC. A 33-mm Watchman occluder device (Boston Scientific, MA, USA) was successfully deployed under general anesthesia in the LAA with a good seal and no leaks. A transesophageal echocardiogram (TEE) detected no mural thrombus. She was discharged with an 8-week course of

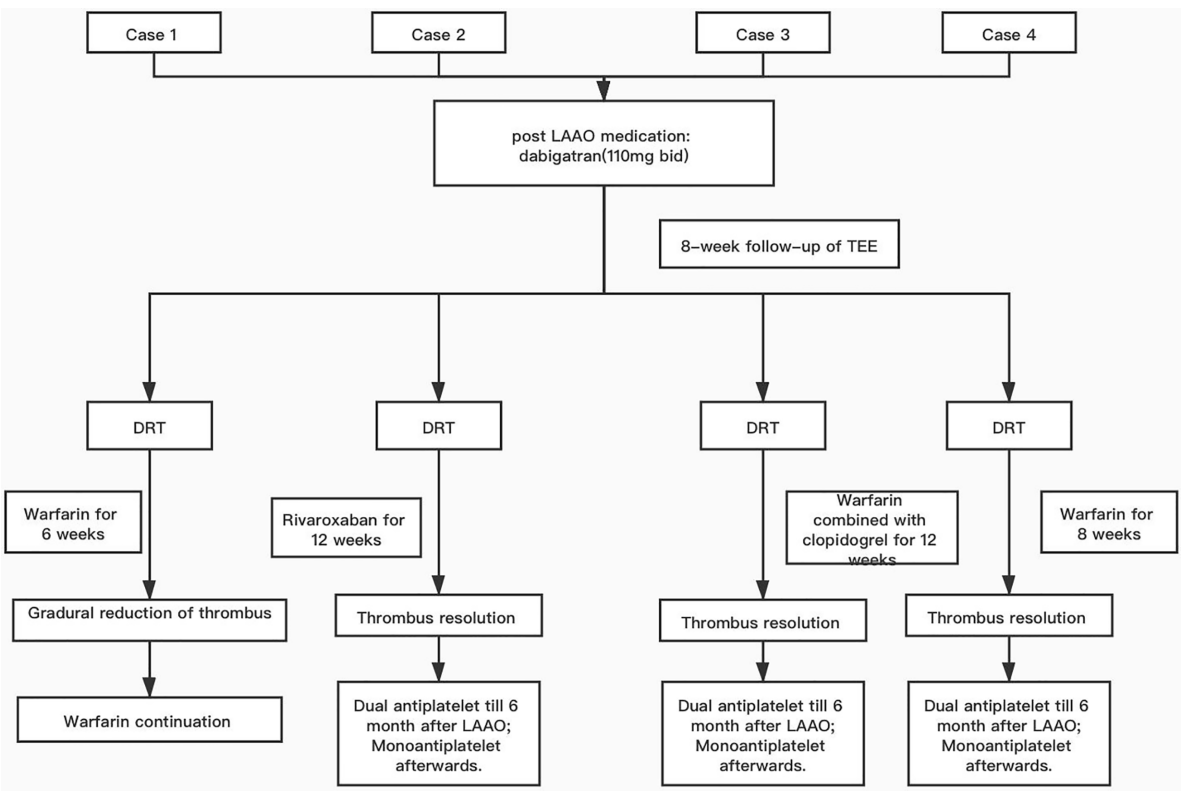

FIGURE 1 | Flow chart of the care process for the presented cases. LAAO, left atrial appendage occlusion; TEE, transesophageal echocardiogram; DRT, device-related thrombosis; dual antiplatelet, 100 mg of aspirin combined with 75 mg of clopidogrel; mono antiplatelet, 100 mg of aspirin only.

dabigatran (110 mg b.i.d.) and a follow-up TEE to assess cardiac function and LAAC device positioning, which might affect further anticoagulation strategies. A proton pump inhibitor (PPI; rabeprazole, 10 mg b.i.d.) was prescribed to reduce the risk of gastrointestinal bleeding. A follow-up TEE, performed 6 weeks later, indicated that thrombus had formed on the occluder surface (**Figure 2**, a1). The physicians decided to switch from dabigatran to warfarin (INR, 2.0–3.0) for anticoagulation. Six weeks later, a TEE revealed the gradual reduction of thrombosis formation on the occluder (**Figure 2**, a2), and physicians made an informed decision to continue on warfarin.

Case 2

A 66−year−old female patient (weight, 65 kg; height, 156 cm; BMI, 26.7 kg/m^2) visited our clinic for further evaluation after having an ischemic stroke. She reported recurrent palpitations and chest pain for 2 years. The ECG demonstrated typical AF patterns with irregular RR intervals and no discernible, distinct P waves. She had a history of hypertension with currently uncontrolled high blood pressure. Paroxysmal AF was diagnosed, and OAC treatment with warfarin was initiated (the CHA_2DS_2-VASc score is 5). After medication with OAC, she suffered upper gastrointestinal bleeding in the following year. She was then transferred to a tertiary cardiology center for a LAAC operation, which provides stroke prevention in patients with nonvalvular AF who are eligible for OAC therapy. As the LAA and left atrium were free of thrombosis, the LAAC operation was performed, and a 30-mm Watchman occluder device (Boston Scientific, MA, USA) was successfully deployed under general anesthesia in the LAA with a good seal and no leaks. She was discharged with an 8-week course of dabigatran (110 mg b.i.d.) for anticoagulation and a follow-up TEE for the assessment of DRT. In consideration of the gastrointestinal bleeding, she was medicated with PPI (rabeprazole, 10 mg b.i.d.). Six weeks after implantation, a TEE revealed thrombosis in the anterolateral surface of the occluder (**Figure 2**, b1). In consideration of warfarin-induced upper gastrointestinal bleeding, the anticoagulation strategy switched from dabigatran to rivaroxaban (15 mg q.d.) due to the appearance of thrombosis, and another TEE was scheduled for 12 weeks later. Under the effective anticoagulation, the new TEE showed a total elimination of thrombosis (**Figure 2**, b2). She suffered no bleeding event after the anticoagulation strategy change in the following 2 years.

Case 3

A 66-year-old man (weight, 87 kg; height, 170 cm; BMI, 30.1 kg/m^2) was referred to our cardiovascular center for the management of a recent ischemic stroke complicated by permanent AF despite receiving antiplatelet therapy with gastrointestinal hemorrhage transformation. A 24-h Holter monitor displayed a dominant rhythm as AF. His medical history included hypertension and coronary heart disease treated with nifedipine controlled-release tablets (30 mg q.d.) and clopidogrel (75 mg q.d.). His CHA_2DS_2-VASc score was 5, in the high-risk category of thrombosis and his HAS-BLED score was 5, in the category of high bleeding risk. Although there was no strong case against OAC use, neurologists indicated a high risk of cerebral bleeding under anticoagulant therapy. Upon confirming no thrombosis in the LAA and left atrium, a LAAC operation was successfully performed on the patient using a 33-mm Watchman occluder device (Boston Scientific, MA, USA) that was placed accurately on the LAA with no immediate complications. The patient was discharged, and anticoagulated with dabigatran (110 mg b.i.d.) for 8 weeks was

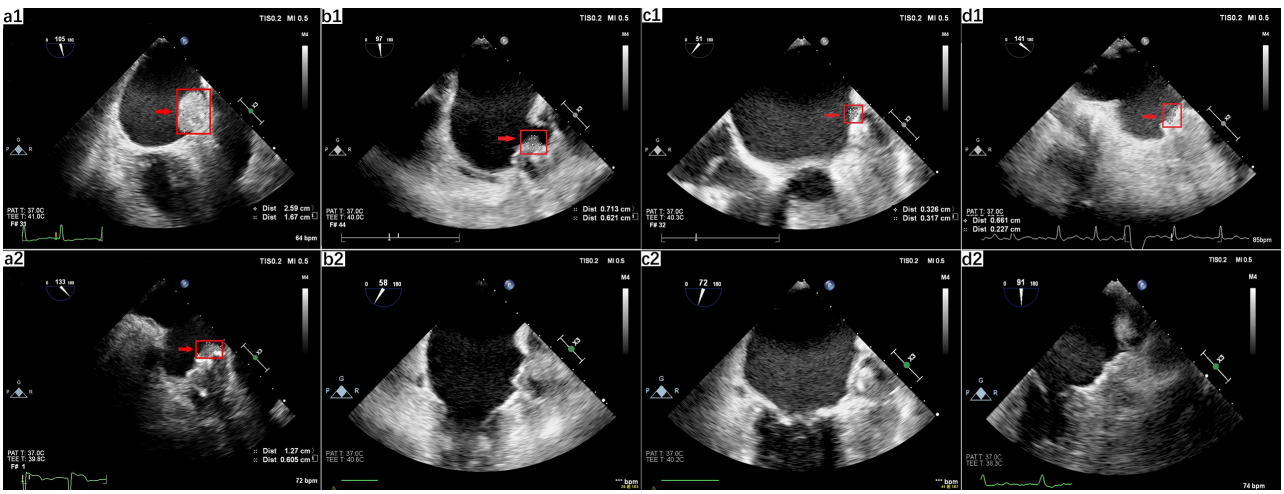

FIGURE 2 | Left atrial appendage occlusion-related thrombus anticoagulated with dabigatran. (a) TEE 2-dimensional view displayed thrombosis (2.59 cm × 1.67 cm) on the surface of the occluder (a1). After six weeks of warfarin treatment, a regression of the thrombosis was shown (1.27 cm × 0.605 cm), which confirmed the diagnosis of device-related thrombosis (a2). (b) TEE revealed a thrombus (0.713 cm × 0.621 cm) on the anterolateral surface of the occluder (b1) and the total disappearance of the thrombus after initiation of anticoagulation therapy with rivaroxaban (b2). (c) TEE check-up revealed abnormal hemodynamics in the LAA, identified as thrombosis (0.713 cm × 0.621 cm) (c1), and total elimination of DRT after 3 months following anticoagulation treatment with warfarin (c2). (d) TEE revealed a thrombus (0.661 cm×0.227 cm) on the surface of the occluder (d1) and the total disappearance of the thrombus after the initiation of anticoagulation therapy with warfarin (d2). Red arrows indicate the thrombosis.

combined with PPI (rabeprazole, 10 mg b.i.d.). Two months later, a TEE check-up revealed abnormal hemodynamics in the LAA, suspected as thrombosis (**Figure 2**, c1). The antithrombotic regimen was switched from dabigatran to warfarin (3.75 mg q.d.) within the therapeutic range (INR, 2.0–3.0), combined with clopidogrel (75 mg q.d.). A further TEE was performed to confirm the resolution of DRT after 3 months following anticoagulation treatment (**Figure 2**, c2). Warfarin was stopped, and the patient resumed dual antiplatelet therapy for six more months followed by life-long aspirin. No transient ischemic attacks (TIA) or bleeding related complications occurred in the follow-up examinations.

Case 4

A 78-year-old man (weight, 78 kg; height, 173 cm; BMI, 26.0 kg/m^2) suffered a transient ischemic attack (TIA) with clinical presentation as hemiplegia and slurred speech for 5 months. The 24-h Holter monitor displayed paroxysmal AF. The patient had an extensive medical history, including coronary artery disease (CAD), arteriosclerosis obliterans, hypertension, and chronic kidney disease (CKD III). The estimated glomerular filtration rate (eGFR) was calculated as 45 by the modified MDRD equation and was diagnosed as stage III renal dysfunction. He received dabigatran (110 mg b.i.d.) for stroke prevention, and routine urine tests revealed hematuria after anticoagulation initiation. His CHA_2DS_2-VASc score was 6 (hypertensive, aged 75 years, and suffering from transient ischemic attacks and vascular disease), and his HAS-BLED score was 6 (hypertension, abnormal renal function, stroke, bleeding, elderly, and drugs). Due to his unsuitability for long term anticoagulation and the high risk of stroke, he was transferred to the cardiology center for further analysis of AF and consideration of LAAC. A 30-mm Watchman occluder device (Boston Scientific, MA, USA) was successfully deployed under general anesthesia in the LAA with a good seal and no leaks. It was confirmed by a TEE that there was no thrombosis in the LAA. He was discharged with an eight-week course of dabigatran (110 mg b.i.d.) for post-implantation anticoagulation treatment, followed by dual antiplatelet therapy (aspirin, 100 mg q.d.; clopidogrel, 75 mg q.d.) for 6 months and aspirin (100 mg q.d.) for life. The routine follow-up TEE for the LAAC device position after 2 months revealed thrombus on the surface of the occluder (**Figure 2**, d1). He was switched from dabigatran to warfarin with a target INR of 2.0–3.0 for prolonged anticoagulation, followed by a repeat TEE scheduled for 8 weeks later. TEE check-ups later revealed the total disappearance of the abnormal thrombosis with the final diagnosis refined as DRT (**Figure 2**, d2). No TIA and bleeding-related complications occurred. Warfarin was stopped and dual antiplatelet therapy (100 mg of aspirin and 75 mg clopidogrel) was initiated instead.

DISCUSSION

To the best of our knowledge, this is the first report to investigate thrombosis formed on LAA devices associated with dabigatran exposure in patients undergoing percutaneous LAAC. There was a total disappearance of the abnormal thrombosis under anticoagulation conversion from dabigatran to rivaroxaban and warfarin. Our findings indicate that dabigatran is less effective than warfarin and rivaroxaban in reducing thrombosis after LAAC procedures. Our report indicates that postoperative DRT may still take place despite the use of dabigatran.

Antithrombotic Therapy for Postoperative Care

Previous animal test results showed that device endothelialization might occur over the LAA surface and extend over the adjacent endothelium following occluder implantation (Bass, 2010; Schwartz et al., 2010). In our review, the occurrence of thrombosis on the novel Watchman device is thought to be more frequent in the first few weeks after implantation and to decline with complete endothelialization of the device surface. According to current guidelines, it is recommended that an intensive course of anticoagulation with NOAC is given to patients ineligible for warfarin to facilitate device endothelialization, followed by dual antiplatelet therapy for six months and then lifelong aspirin (Glikson et al., 2019).

Risk Factors of Thrombosis Formation After LAAC Operation

In our study of the four patients with DRT, routine follow-up TEE identified post-procedure thrombosis formation. Given the common use of LAAC operations in patients with intolerant bleeding risk under OAC, DRT leaves both patients and physicians with a dilemma: it provides an iatrogenic indication for therapeutic anticoagulant therapy and additional TEE check-ups. Many risk factors might contribute to DRT including procoagulant patient-specific factors with a high risk of thrombosis, device implantation specific factors, and inappropriate anticoagulation (Kaneko et al., 2017). Notably, incomplete closures with peri-device leakage were associated with thromboembolic events (Kanderian et al., 2008). It was likely that residual flow around the device into a stagnant LAA pouch might contribute to turbulent blood flow and enhance platelet adhesion and clot formation (Saw et al., 2017). In our four cases, TEE imaging displayed a good seal and no leaks for occluders inserted on the LAA which ruled out device implantation specific factors. The mechanism of thrombosis formation after LAAC operations was more likely involved with high-risk procoagulant features and inappropriate anticoagulation. Also, the deployed device size may be another probable contributor to DRT. A previous study reported that the device size was larger in patients with development of thrombus (29.3 ± 3.8 mm vs. 25.7 ± 3.2 mm, respectively) after the Watchman device implantation (Kubo et al., 2017). The increased risk of thrombus formation may be explained by the larger area of the fabric on the larger device. In our cases, the patients were implanted with a device size of 33 mm, which could add up to the risk of postoperative DRT. However, we think that anticoagulation with dabigatran played a principal role, since complete thrombus resolution was observed after switching to alternative anticoagulation therapy.

Post-LAAC Anticoagulation With NOAC for DRT Prevention

After successful Watchman implantation, a post-thrombotic regimen with NOAC is considered as a substitute for patients

who are unable to tolerate a short duration of warfarin until complete endothelialization of LAA devices (Reddy et al., 2017). Effective and safe therapy with warfarin requires continuous monitoring of prothrombin time (PT) and INR levels to adjust the dose of warfarin (Numao et al., 2017). Warfarin has a narrow therapeutic window with an INR in the range of 2.0–3.0, and many factors can influence the warfarin dosing algorithm including patient characteristics such as body mass index (BMI), age, comorbidities, concomitant drugs, and diet, as well as genetic variants for warfarin metabolism *via* cytochrome P450 (CYP) 2C9 and genetic differences in recycling vitamin K through vitamin K epoxide reductase (VKORC1) (Verhoef et al., 2014; Gage et al., 2017; Drozda et al., 2018). Many randomized clinical trials (RCT) demonstrated that NOAC was superior to warfarin in efficacy and safety and NOACs seemed to be more effective and safer for short period anticoagulation compared with warfarin for patients post LAAC operation (Connolly et al., 2009; Reddy et al., 2017). However, there are currently no comparisons of clinical efficacy and safety with NOACs for patients undergoing LAAC operations.

Thrombosis on Closure Devices Anticoagulated With Dabigatran

Dabigatran, a direct inhibitor of thrombin, has been shown to be an alternative anticoagulant for patients intolerant of warfarin in the prevention and treatment of thromboembolic disease (Connolly et al., 2009). In these four cases, the follow-up TEE imaging displayed thrombosis with DRT; therefore, anticoagulant adjustment was needed to treat the thrombus. At present, there is no relevant literature about the mechanism for dabigatran medication increasing thrombosis risks post LAAC operation. One clinical study demonstrated that dabigatran medication could increase platelet reactivity by enhancing thrombin receptor density on thrombocytes, contributing to increased risk of myocardial infarction (Franchi et al., 2016). Dabigatran-enhanced platelet reactivity induced by the thrombin receptor activating peptide is specific to thrombin-induced platelet activation (Reilly et al., 2014; Yau et al., 2014). This might be one reason for the occurrence of DRT after a LAAC operation.

Like other blood-contacting medical devices, the occluder components trigger thrombosis formation *via* activation of the intrinsic pathway. It is possible that after LAAC higher than the conventionally used doses of dabigatran (i.e., 100 mg b.i.d.) may be required to prevent DRT. Also, some studies indicate that standard dosing regimens may be associated with lower dabigatran plasma concentrations in obese patients because of higher volumes of distribution (Yau et al., 2014). BMI of >25 was the cut-off point according to the World Health Organization for obesity (Haschke et al., 2016). This might lead to a reduction in the anti-thrombosis effect of dabigatran and an increase in the incidence of DRT (Lucijanic et al., 2020). One probable explanation for our four cases with closure device-related thrombosis anticoagulated with dabigatran was the suboptimal drug dosage levels which potentially increased the risk of thrombosis.

Another probable mechanism for attenuated anticoagulation might be a dabigatran/PPI interaction leading to decreased dabigatran plasma concentration. The bioavailability of dabigatran etexilate is pH-dependent, and co-administration with PPI could increase gastric pH levels, which might decrease the dissolution of dabigatran etexilate (Schnierer et al., 2020). Three cases in our study were in concomitant medication with PPI after the LAAC operation.

The common genetic variants of CES1 and ABCB1 have been identified to potentially account for the interindividual variations in dabigatran plasma levels which could lead to varied anticoagulation therapeutic responses (Sychev et al., 2020). It has been proven that the single nucleotide polymorphism (SNP) in the CES1 gene (rs2244613) could alter dabigatran metabolism, leading to lower trough concentrations and increasing thrombosis risks (Sychev et al., 2020). However, we did not investigate the two associated genetic variants in these four cases.

Thrombus Reversal After Transferring to Rivaroxaban and Warfarin

After the confirmation of thrombus, we switched to rivaroxaban and warfarin for anticoagulation, leading to a gradual reduction of thrombosis. In contrast to the anticoagulation mechanism of dabigatran, rivaroxaban is a factor X inhibitor and selectively inhibits FXa with a rapid onset of action, which was beneficial to the prevention of thrombosis and platelet aggregation (Enomoto et al., 2017). Many clinical trials demonstrated that oral rivaroxaban co-administration with antiplatelet therapy could decrease the incidence of thromboembolism with thromboembolic events and deaths due to cardiovascular events, myocardial infarction and stroke (Rubboli et al., 2015).

CONCLUSION

DRT after LAAO has been acknowledged to have a strong correlation with the risks of postoperative stroke and systematic embolic events. Hence, in these cases, the patients are still confronted with neurologic morbidity and mortality. Moreover, the incidence of re-hospitalization and outpatient follow-up visits for adjusting anticoagulation medication might also lead to a waste of medical resources and the added burden of medical expenses. Besides procedural factors including larger device size and deep implantation, which were related to the risk of thrombus formation, novel oral anticoagulant usage such as dabigatran at discharge was also associated with a risk of thrombus formation. The patient BMI and co-administration with PPI should be taken into account during the medication process. The management of such a complication is not standardized, and transferring to rivaroxaban and warfarin might be an alternative anticoagulation strategy. The limitation was the absence of dabigatran related genetic testing for these patients.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/ supplementary material.

ETHICS STATEMENT

Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

XL wrote manuscript and performed literature search and review. XZ provided case and corrected manuscript. QJ provided case and corrected manuscript.

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CASE REPORT
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Prothrombin Complex Concentrate in Liver Transplant Surgery: Correction of Therapeutic Anticoagulation and the Coagulopathy of End-Stage Liver Disease: Case Series

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Suggested treatment for active bleeding or invasive procedure prophylaxis has been described in the setting of end-stage liver disease (ESLD) in patients not receiving anticoagulation, and has included fresh frozen plasma (FFP), prothrombin complex concentrates (PCC), platelets, and cryoprecipitate. Today, the therapy for pharmacologically anticoagulated patients with ESLD presenting for liver transplant surgery remains controversial, poorly studied, and physician-dependent. We observed a variety of treatments administered at initiation of liver transplantation to correct acquired coagulopathy at our leading transplant center and present these cases. Three patients receiving preoperative therapeutic anticoagulation with warfarin for acute deep venous thrombosis and/or atrial fibrillation were transfused PCC, FFP, and/or cryoprecipitate for liver or liver-kidney transplant surgery. No thrombotic complications occurred, and one patient required reoperation for hemorrhage. We report data from these cases including estimated blood loss, presence of complications, duration of ICU stay, and length of hospitalization. Perioperative orthotopic liver transplant hematologic management and a review of relevant literature is presented.

Keywords: liver transplant anesthesia, liver transplant anesthesiology, prothrombin complex concentrate (PCC), Kcentra, 4-factor prothrombin complex concentrate, end stage liver disease (ESLD), transfusion - alternative strategies

INTRODUCTION

Altered coagulation inherent to end stage liver disease (ESLD) can prove difficult to manage in the medical patient, but is particularly challenging in the patient who may urgently undergo liver transplantation (LT). The ESLD population exhibits derangement in traditional coagulation studies often demonstrating a coagulopathic patient, while more modern viscoelastic testing and clinical studies may define a patient as hypercoagulable and at increased risk for thrombosis (Bezinover et al., 2018). Having established a hypercoagulable profile, many subjects who develop thromboses require therapeutic anticoagulation preoperatively. Fresh frozen plasma (FFP) or prothrombin

complex concentrates (PCC) as treatment for active bleeding or in preparation for invasive procedure have been described for patients with ESLD not receiving anticoagulation. However, the recommended correction of therapeutic anticoagulation in the ever-more-common anticoagulated patient with ESLD undergoing liver transplantation is not well-described in literature and is varied and physician-dependent in practice (Drebes et al., 2019) as is observed in this case series from our leading transplant center.

PCC is a pooled plasma product, and formulations contain three (II, IX, X) or four (II, VII, IX, X) vitamin K-dependent coagulation factors, proteins C and S, and often heparin (Verbeek et al., 2018; Drebes et al., 2019). PCC factor concentrations are 25 times greater than in FFP, more rapidly and effectively correcting international normalized ratio (INR), with low volume administration that is completed in 10 min (Franichini and Lippi, 2010; Intagliata et al., 2017; Drebes et al., 2019). PCC is easily reconstituted from the lyophilized form and requires no blood group specificity (Cappabiana et al., 2016). PCC likely does not increase the risk of thromboses, and has lower risk of viral transmission and volume overload than FFP (Bezinover et al., 2018; Drebes et al., 2019). Dosing is typically based on INR, however fixed-dosing strategies have been investigated (Leissinger et al., 2008; Dane et al., 2019). KCentra is the only Food and Drug Administration (FDA)-approved four-factor PCC in the United States, and its approved indication is for the reversal of acquired coagulopathy from vitamin K antagonists (VKA) during acute major bleeding or urgent invasive procedure (Sritharan and Triulzi, 2018).

Our tertiary hospital performs over 100 orthotopic LT surgeries each year. Preoperative and postoperative management by surgical and intensive care teams is most often guided by traditional coagulation studies: prothrombin/international normalized ratio (PT/INR), partial thromboplastin time (PTT), platelet count, and fibrinogen level. Intraoperative management includes viscoelastic testing along with cues from patient condition and perceived surgical field bleeding tendency as reported by the surgeon. Balanced transfusion strategies are consistently observed, but therapy to reverse pharmacologic anticoagulation is varied in these three cases of anticoagulation reversal.

The patients presented were treated with PCC monotherapy or in combination with FFP, and one patient was treated with FFP and cryoprecipitate **Table 1**. Each strategy was selected collaboratively by the anesthesiology and surgical teams. The cases are presented to demonstrate varied approaches to treatment of acquired coagulopathy in liver transplant patients. Informed consent was obtained for each case presented.

Case Presentations

Case A

A 59-year-old female patient with autoimmune hepatitis-related ESLD was diagnosed with bilateral lower extremity deep venous thromboses (DVT) 1 month prior to liver transplantation and pharmacologically anticoagulated with warfarin with preoperative INR of 2.4. The patient was treated with 1,518 U PCC at initiation of surgery, with resultant normalized extrinsic pathway thromboelastometry (EXTEM) portion of viscoelastic testing and clinically improved surgical field. Traditional coagulation studies were continued for comparison and INR was 1.6 30 min after administration of PCC and 2.4 postoperatively. Intraoperative estimated blood loss (EBL) was 500 ml, and no significant bleeding was evident postoperatively. The patient received no blood products intraoperatively and 2 U of FFP postoperatively. The patient was discharged from the hospital on postoperative day (POD) 5 without postoperative complications.

Case B

A 60 year old male patient with nonalcoholic steatohepatitis (NASH) and hepatitis C cirrhosis, and atrial fibrillation (AF) on warfarin presented for LT with preoperative INR of 3. The patient was treated with FFP and cryoprecipitate at initiation of surgery. The following were transfused intraoperatively: 14 U red blood cells (RBC), 16 U FFP, 2 platelet pools, and 4 U cryoprecipitate with an EBL of 10 L. Immediate postoperative INR was 1.3. Due to hemorrhagic shock, the patient returned to the operating room POD 0 where retroperitoneal bleeding was identified, and there was an additional 1.5 L of blood loss. During the subsequent surgery, the patient was transfused 5 U RBC, 2 platelet pools, 3 U FFP, and 4 U cryoprecipitate. Acute kidney injury with peak creatinine of 3.3 recovered, and no other complications were present postoperatively. The patient was discharged on POD 15.

Case C

A 67-year-old male patient with NASH cirrhosis, end-stage renal disease (ESRD) on hemodialysis (HD), coronary artery disease (CAD) with stent, who was treated with warfarin for deep venous thrombosis (DVT) and AF with preoperative INR of 2

TABLE 1 | Patient characteristics and therapies.

	Case A	Case B	Case C
Anticoagulation	warfarin	warfarin	warfarin
Reason for anticoagulation	DVT	AF	DVT, AF
Preoperative MELD score	21	22	30
Estimated surgical blood loss (L)	0.5	10, 1.5*	8
RBC transfusion required	no	yes	yes
Duration of ICU stay (days)	1	2	2
Duration of hospital stay (days)	5	15	12
Complications	none	Reoperation, acute kidney injury	none

Estimated blood loss for second operation.

underwent liver-kidney transplantation. Initial rotational thromboelastometry (ROTEM) identified prolonged clotting time. The patient received 2,112 U PCC and 2 U FFP at the start of surgery. Postoperative INR was 2.6. EBL was 8 L, and 12 U RBC, 7 U FFP, 3 platelets pools, and 5 U cryoprecipitate were transfused. The patient was discharged POD 12 without postoperative complication.

DISCUSSION

Coagulation in ESLD is dysregulated with alterations occurring at each level of the coagulation pathway (Bezinover et al., 2018; Verbeek et al., 2018; Drebes et al., 2019). There is a decreased concentration of liver-dependent procoagulant factors, while anticoagulant factors proteins C&S and ATIII are also decreased (Bezinover et al., 2018). The reduction in these competing factors is not balanced (Verbeek et al., 2018). Liver-independent factors VIII, vWF, and plasminogen activator inhibitor 1 all increase. Decreased concentration of ADAMTS-13, which deactivates vWF, leads to more active vWF. Thrombocytopenia is impacted by splenic sequestration of platelets, and platelet function is reduced (Bezinover et al., 2018; Verbeek et al., 2018). Fibrinolytic balance is likewise altered with a decrease in liver-dependent factors and an increase in liver-independent factors. Clots in ESLD patients form more slowly, are less permeable and less readily lysed, which is likely related to oxidation of the fibrinogen molecule (Bezinover et al., 2018; Verbeek et al., 2018). Leakage of portal endotoxin from portal to systemic circulation is known to occur in cirrhotic patients and causes endothelial dysfunction by inflammation and oxidative stress (Verbeek et al., 2018).

Derangement in conventional coagulation studies that measure the intrinsic and especially the extrinsic pathway can be expected in ESLD patients (Bezinover et al., 2018; Verbeek et al., 2018). INR is not accurately reflective of coagulation status in ESLD, but instead is more valuable for its prediction of mortality when used for model for end-stage liver disease (MELD) or Child-Turcotte-Pugh score calculation. Viscoelastic testing provides a clearer picture of coagulation status (Northup and Reutemann, 2018).

The net result of all of these changes to the coagulation system is a precarious balance with little reserve, which may be easily tipped in either direction resulting in thrombosis or excessive hemorrhage. Thus, patients with ESLD undergoing LT are at increased risk for bleeding and thromboembolic events (Bezinover et al., 2018). Spontaneous bleeding is uncommon, and bleeding events are more likely to be procedure-related (Verbeek et al., 2018; Drebes et al., 2019). Due to decreased hemostatic reserve, surgical bleeding is more likely to result in decompensation. Intraoperative transfusion of blood products is predictive of survival after LT, and the need for transfusions during LT has declined over time (Bezinover et al., 2018; Verbeek et al., 2018).

Thrombotic Risk in Chronic Liver Disease

Chronic liver disease (CLD) patients are at greater risk for arterial and venous thromboembolic (VTE) events. Hospitalized CLD patients' risk for VTE is 0.5–8.2% (Verbeek et al., 2018). Post-LT thrombotic complications are also common with VTE discovered in 5–10%, portal vein thrombosis (PVT) in 2%, and hepatic artery thrombosis (HAT) in 6% of patients. Patients with NASH cirrhosis have higher risk for development of pulmonary embolism (PE), DVT, and PVT, while those with autoimmune disease have greater risk for PVT with higher levels of fibrinogen and tissue factor identified postoperatively. African Americans have greater risk for VTE but lower risk PVT. Hypercoagulability is evident in each phase of transplant care, and does not resolve immediately after transplant (Verbeek et al., 2018).

Venous Thromboembolic Prophylaxis in End Stage Liver Disease

Prophylactic anticoagulation to prevent DVT in hospitalized patients with ESLD is often provided with heparin or low-molecular-weight-heparin (LMWH), but activity of these medications is difficult to monitor, with an expected baseline elevation of PTT. Forty-four percent of hospitalized patients with CLD receive pharmacologic prophylaxis, a statistic that is increasing. Prophylaxis appears to be safe, though literature in this specific population is limited. Among postoperative patients receiving DVT prophylaxis who have undergone resection for hepatocellular carcinoma, the preoperative presence of esophageal varices (EV) has been identified as the only risk factor for bleeding complications. It is recommended that hospitalized patients with CLD receive DVT prophylaxis with heparin or LMWH if there is no contraindication, and alternatively sequential compression devices should be used (Verbeek et al., 2018).

Therapeutic Anticoagulation in Chronic Liver Disease

Therapeutic anticoagulation for VTE in ESLD patients has been described with the use of several classes of anticoagulant. VKAs may be used but have a narrow therapeutic index with considerations including diet, monitoring, and underlying disease. Pharmacokinetics are altered in CLD patients due to changes in absorption, volume of distribution, and decreased protein binding. Baseline INR elevation is often present and there may be inter-laboratory variability of testing. Additionally, increasing the INR impacts the MELD score (Verbeek et al., 2018).

Therapeutic anticoagulation in ESLD with direct-acting oral anticoagulants (DOAC) has been limitedly described, and larger trials of these medications excluded cirrhotic patients (Verbeek et al., 2018). Intagliata et al. demonstrated similar risk for bleeding events among 39 cirrhotic patients treated for 3 years with DOAC or warfarin. No specific risk factors for bleeding events were identified among these groups (Intagliata et al., 2016).

Factor Xa inhibitors show similar rates of complication to controls in Child-Turcotte-Pugh classes A and B, data which may not be applicable in the transplant population (Verbeek et al., 2018). Reversal of this class with PCC and other products is not well-studied. Dialysis or idarucizumab, which has been described as safely administered during LT, may be considered in the case of dabigatran (Intagliata et al., 2017; Verbeek et al., 2018). Andexanet is approved by the FDA for reversal of rivaroxaban and apixaban but was not studied in cirrhotic patients (Intagliata et al., 2017).

Postoperative HAT prophylaxis with nonsteroidal anti-inflammatory drugs (NSAIDs) may be considered with consideration for risk of bleeding from EV and the risk of acute renal failure. NSAIDs appear to be safe therapy in ESLD patients with EV after coronary artery stenting (Verbeek et al., 2018).

There remains no agent of choice for anticoagulation and its selection should be determined on an individual basis. Conservative dosing is recommended with any anticoagulant therapy in the ESLD population (Verbeek et al., 2018).

Treatment of End Stage Liver Disease Coagulopathy

The current standard of care to remedy coagulopathy in ESLD patients who are not receiving systemic anticoagulation and who have active bleeding or planned invasive procedure is FFP, with suggested volume of at least 15 ml/kg (Northup and Reutemann, 2018; Drebes et al., 2019). PCC may alternatively be considered. In all clinical practice, PCC is used most frequently for reversal of VKAs, which cause major bleeding complications in 1.1–1.5% of patients annually (Franichini and Lippi, 2010).

Most studies show no clinically meaningful reduction in bleeding risk to preprocedural transfusion of FFP and rarely is INR goal met in non-anticoagulated patients. Large volume, risk for transfusion-related acute lung injury (TRALI), and volume overload have been reported with FFP administration. Northup et al. do not recommend the use of FFP use for INR reduction preprocedure in ESLD patients due to the risk for life-threatening complications of transfusion and the lack of supportive data for clinically reduced bleeding (Northup and Reutemann, 2018).

Prothrombin Complex Concentrate for Reversal of Anticoagulation in Other Surgical Populations

Preprocedure anticoagulation with warfarin is well-described in patients with atrial fibrillation undergoing heart transplantation. Both FFP and PCC have been used for corrective therapy (Salerno et al., 2017). Kantorovich et al. demonstrated in a retrospective cohort study that used low-dose PCC for preoperative heart transplant warfarin correction with successful reduction of INR and transfusion of FFP, and no greater risk of thromboembolism (Kantorovich et al., 2015).

Arachchillage *et al.* studied 344 patients taking warfarin, rivaroxaban, and apixaban with major bleeding events, most commonly intracranial hemorrhage, who received treatment with PCC. Treatment was considered to be equally successful in 72.5–77.6% of patients from each of the three anticoagulant therapy groups. Thromboembolic events were statistically similar across the groups at 2.5–5.1%. There was no difference in transfusion of other products among the three groups (Arachchillage et al., 2019). Arachchillage also retrospectively evaluated patients undergoing cardiac surgery whose anticoagulation therapy was treated with PCC or FFP and found among the PCC group significantly less cardiac failure related to circulatory overload; no difference was reported in 30 days mortality, kidney injury, or thrombotic events. However, the same study reported a statistically significant increase in blood loss at 24 h post-surgery (1,575 *vs.* 1,213 ml) (Arachchillage et al., 2016).

Thrombotic Risk of Prothrombin Complex Concentrate

Previously there was concern for risk of thrombotic complications with PCC, however these events appear to be related to high or repeated doses of PCC. Drebes et al. did not show an increased rate of thrombotic complication with the use of slightly less than the recommended lowest dose for reversal of vitamin K antagonist of 25 IU/kg with a median dose of 22 IU/kg in 105 patients given PCC for preprocedure prophylaxis or bleeding event (Drebes et al., 2019). Other acquired coagulopathies have been treated with PCC and are described in the literature. Sritharan et al. performed retrospective study of 213 patients with bleeding events who received PCC for correction of coagulopathy associated from anticoagulation with warfarin (40%), apixaban (21%), rivaroxaban (23%) therapies and the remaining 16% from cirrhosis, disseminated intravascular coagulation (DIC), or antiplatelet medication. There was lower mortality among the coumadin, rivaroxaban, apixaban groups; and no benefit in the cirrhosis, DIC, or antiplatelet groups. They reported a significantly increased risk of DVT and stroke in the cirrhosis group and a moderate increase for the rivaroxaban group (Sritharan and Triulzi, 2018). As mentioned in the above section, Kantorovich and Arachnillage performed separate studies on diverse populations who received PCC and reported no increase in thrombotic events.

Intraoperative intracardiac thrombus (ICT) or PE occurs in 1–6% of LT patients and carries a high mortality, with actual incidence likely greater with increased identification as transesophageal echocardiography use becomes more common (Dalia et al., 2017). Risk factors for intraoperative thrombotic events include preoperative VTE, history of transjugular intrahepatic portosystemic shunt (TIPS) procedure, use of veno-veno bypass, and use of PA catheter or antifibrinolytics (Verbeek et al., 2018). In a retrospective analysis of 30 patients, 10 of whom received PCC during preoperative hospitalization, Misel et al. reported five patients with intraoperative ICT. An association was determined to include preoperative PCC dose within 14 days of surgery, a history of GI bleeding, and possibly preoperative dialysis treatment. The median time for PCC administration before surgery was 4–6.5 days and usually greater than one dose was given (Misel et al., 2018).

The literature available provides reassuring data. PCC appears to be safest in patients with ESLD who are anticoagulated, with the exception of moderately increased thrombotic risk in patients receiving rivaroxaban. Particular attention to risk for thrombotic complication must be considered if treating patients with cirrhosis-related coagulopathy not undergoing LT, or if electing to use PCC during hospitalization in the several days leading to LT.

Prothrombin Complex Concentrate Use in Liver Transplantation

One year survival after LT is reduced in patients who receive more blood products intraoperatively, though causality is not concluded as many factors contribute to the need for transfusion. Transfusion of blood products has been associated with an increased risk of postoperative infection, respiratory complications, duration of hospital stay, and graft failure. Red blood cell (RBC) and FFP transfusion has been associated with increased age, PT, length of surgery, and blood urea nitrogen (BUN), presence of cirrhosis, or decreased serum albumin concentration. Other factors associated with a greater need for FFP were preoperative encephalopathy and elevated AST. Platelet transfusion is more likely with increased age, length of surgery, BUN, and PT, or decreased serum albumin (Kasraian et al.,).

Villalpando et al. reported that PCC given at the beginning of LT to correct non-acquired ESLD coagulopathy in 39 patients reduced the EBL by about 500 ml and reduced the need for FFP transfusion. Complications were not reported (Leal-Villalpando et al., 2016). In another retrospective cohort study, Srivastava et al. evaluated the utility of PCC as first line therapy in LT coagulopathy. PCC administration was associated with less RBC and FFP transfusion, and less incidence of hemorrhagic complication requiring surgical re-exploration. Groups had the same need for postoperative ventilation, HD, length of stay, occurrence of thrombosis, or thromboembolism. Some significant differences between the two groups' characteristics were present and those receiving PCC were often in worse health with higher MELD, creatinine, INR, and lower hemoglobin, platelet level, and/or serum fibrinogen level. Propensity matching grouped 60 similar pairs (Srivastava et al., 2018).

Combination Therapy/Future Direction for Liver Transplantation

A retrospective, observational analysis by Kirchner et al. in Germany, which studied fibrinogen concentrate (FibC) and PCC together as a combined first-line, ROTEM-guided hemostatic therapy for coagulopathy in LT found no increase in the risk of thrombotic or ischemic events. Significantly fewer RBC units were transfused in the factor concentrate group when groups with and without coagulation factor concentrate were compared (Kirchner et al., 2014). A competing study found no difference in transfusion requirement in patients given PCC and FibC during LT (Colavecchia et al., 2017).

CONCLUSION

Acquired coagulopathy was treated in three liver transplant patients with PCC monotherapy, in combination with FFP, or alternatively with FFP and cryoprecipitate. The cases were presented to illustrate the present variable therapy utilized for anticoagulated patients undergoing LT.

Both case A and case C received PCC to treat acquired coagulopathy related to warfarin therapy. However, only case A received no blood products intraoperatively. It is important to consider that case C was a more complex liver-kidney transplant surgery, in a patient with additional comorbidities of CAD, ESRD, and AF, though this should not suggest causation.

Transfusion requirement was less for the patient treated with PCC monotherapy, however the surgery was less complex and there were fewer significant comorbidities present at baseline. The only patient to require reoperation in this limited sample was the patient who was treated initially with FFP and cryoprecipitate. Among the patients, acute kidney injury (AKI) was the only reported postoperative complication.

Further clinical trials are warranted to determine safe, optimal therapy for the coagulopathic patient with ESLD urgently undergoing LT, and therapy continues to be varied in clinical practice. PCC appears to be an effective treatment for acquired coagulopathy in patients with ESLD undergoing LT who have received pharmacologic anticoagulation, with little or no apparent increase in rate of complications. In patients with ESLD-related coagulopathy who are not anticoagulated, it may be reasonable to select PCC as first-line therapy. In this case, there must be careful consideration for possible thrombotic risk noted in patients with cirrhosis treated for bleeding events with PCC who were not undergoing LT, and for those hospitalized patients treated several days prior to LT.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/supplementary material.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JN and AF contributed to writing and editing of the manuscript. All authors contributed to the article and approved the submitted version.

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Ginsenoside Re Treatment Attenuates Myocardial Hypoxia/Reoxygenation Injury by Inhibiting HIF-1α Ubiquitination

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Previous studies have shown an attenuating effect of ginsenoside Re on myocardial injury induced by hypoxia/reoxygenation (H/R). However, the underlying mechanism remains unclear. This study was designed to determine the underlying mechanism by which ginsenoside Re protects from myocardial injury induced by H/R. HL-1 cells derived from AT-1 mouse atrial cardiomyocyte tumor line were divided into control, H/R, and H/R + ginsenoside Re groups. Cell viability was measured by CCK-8 assay. ATP levels were quantified by enzymatic assays. Signaling pathway was predicted by network pharmacology analyses and verified by luciferase assay and gene-silencing experiment. The relationship between ginsenoside Re and its target genes and proteins was analyzed by docking experiments, allosteric site analysis, real-time PCR, and ubiquitination and immunoprecipitation assays. Our results showed that ginsenoside Re treatment consistently increased HL-1 cell viability and significantly up-regulated ATP levels after H/R-induced injury. Network pharmacology analysis suggested that the effect of ginsenoside Re was associated with the regulation of the Hypoxia-inducing factor 1 (HIF-1) signaling pathway. Silencing of *HIF-1α* abrogated the effect of ginsenoside Re on HL-1 cell viability, which was restored by transfection with an HIF-1α-expressing plasmid. Results of the bioinformatics analysis suggested that ginsenoside Re docked at the binding interface between HIF-1α and the von Hippel-Lindau (VHL) E3 ubiquitin ligase, preventing VHL from binding HIF-1α, thereby inhibiting the ubiquitination of HIF-1α. To validate the results of the bioinformatics analysis, real-time PCR, ubiquitination and immunoprecipitation assays were performed. Compared with the mRNA expression levels of the H/R group, ginsenoside Re did not change expression of *HIF-1α* mRNA, while protein level of HIF-1α increased and that of HIF-1α[Ub]n decreased following ginsenoside Re treatment. Immunoprecipitation results showed that the amount of HIF-1α

bound to VHL substantially decreased following ginsenoside Re treatment. In addition, ginsenoside Re treatment increased the expression of GLUT1 (glucose transporter 1) and REDD1 (regulated in development and DNA damage response 1), which are targets of HIF-1α and are critical for cell metabolism and viability. These results suggested that Ginsenoside Re treatment attenuated the myocardial injury induced by H/R, and the possible mechanism was associated with the inhibition of HIF-1α ubiquitination.

Keywords: ginsenoside Re, cardiomyocytes, hypoxia/reoxygenation injury, HIF-1α, ubiquitination

INTRODUCTION

Although coronary blood flow restoration rescues the ischemic myocardium, myocardial ischemia/reperfusion (I/R) may lead to sustained and even irreversible myocardial ultrastructural damage (Chen et al., 2019). Myocardial I/R injury, reported to be a complicated process involving many signaling pathways, results in apoptosis and necrosis of cardiomyocytes and counteracts the effect of reperfusion therapy (Li et al., 2019). At supra-cellular level, even dysfunctional changes in heart muscle without necrosis, such as fatal arrhythmia and cardiogenic shock, may create a danger to the heart (Refaey et al., 2019).

A previous study demonstrated that ginsenoside Re, a component of Panax ginseng, protects cardiomyocytes against H/R injury (Lim et al., 2013); however, the mechanism remains unclear. This study was designed to test whether the underlying mechanism of ginsenoside Re in attenuating myocardial H/R injury was associated with inhibiting HIF-1α ubiquitination.

MATERIAL AND METHODS

Materials

Ginsenoside Re with a purity of 92.3% was purchased from the National Institute of Food and Drug Control (No. 110754-201525). HL-1 cells were gifted by Professor Yanzhong Chang (Hebei Normal University). The hypoxia response element (HRE)-luc plasmid was gifted by Professor Tatsuya Kobayashi (Endocrine Unit, Massachusetts General Hospital, Harvard Medical School). The HA-HIF-1α plasmid was purchased from Addgene. The His-ubiquitin plasmid was gifted by Professor Lingqiang Zhang (State Key Laboratory of Proteomics, Beijing Proteome Research Center, National Center for Protein Sciences Beijing, Beijing Institute of Lifeomics). Anti-Myc and anti-Flag monoclonal antibodies were purchased from Cell Signaling (Danvers, Massachusetts, USA) and Sigma (St Louis, Missouri, USA), respectively. SiRNAs targeting the HIF-1α gene were designed and synthesized by Shanghai GenePharma Co., Ltd.

The HIF-1α siRNA sequence was 5′-UAAUAUCUUC UUUAUUGUCCU-3′.

Cell Culture, Model, and Treatment

HL-1 cells, from a cell line derived from the AT-1 mouse atrial cardiomyocyte tumor lineage, retain the *in vitro* phenotypic characteristics of adult cardiomyocytes (Vicencio et al., 2015) and were cultured with supplemented Claycomb medium comprising 90% Claycomb medium (JRH Biosciences, 51800C-500 ML), 10% fetal bovine serum (Sigma-Aldrich, F2442-500 ML), 100 U/ml:100 μg/ml penicillin/streptomycin (Life Technologies, 15140-122), 0.1 mM norepinephrine (Sigma, A-0937; 10 mM stock), and 2 mM L-glutamine (Life Technologies, 25030-081; 200 mM stock). The HL-1 cells were passaged every two or three days.

The HL-1 cells were divided into control, H/R, and H/R + Re groups. The cells in the control group were maintained under normoxic conditions (21% O_2, 5% CO_2, and 74% N_2) for 19.5 h. The HL-1 cells in the H/R group were cultured in a hypoxic and sugar-free chamber containing 95% N_2 and 5% CO_2 for 18 h and then placed in a normal chamber containing 21% O_2, 5% CO_2, and 74% N_2 for 1.5 h (Vicencio et al., 2015). The HL-1 cells in the H/R + Re group were treated with 100 μM ginsenoside Re in the and otherwise treated the same as the cells in the H/R group.

Cell Viability

Cell viability was measured with Cell Counting Kit-8 (CCK-8) (CK04; Dojindo, Japan) according to the manufacturer's instructions. Briefly, 10 μl of CCK-8 solution was added to each well (100 μl medium) and incubated for 1 h at 37°C, and then, the absorbance was measured at 450 nm in a microplate reader (BL941; Berthold, Germany).

ATP Level

The ATP levels were quantified using a commercial kit (A095-1; Nanjingjiancheng, Nanjing, China) according to the manufacturer's instructions.

Network Pharmacology Prediction

The drug targets predicted by BATMAN-TCM were filtered using a Perl script with a confidence score cut-off of 3.0. These selected drug targets were mapped to human and rat orthologs. Pairwise ortholog mapping files ("human to rat") were downloaded from the InParanoid database (Release 8.0) (Sonnhammer and Östlund, 2014). The statistics from the KEGG pathway enrichment analysis of the selected drug targets were generated using the clusterProfiler package in R.

Docking Analysis

The Schördinger Maestro package was used to perform a dock analysis. The 2D ligand structure of the ginsenoside Re molecule was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov)and

prepared using LigPrep. VHL and HIF-1α protein structure was modeled with I-TASSER and constructed following the Protein Prepare Wizard workflow in the Maestro package (Roy et al., 2010). All chains of the structure were used to prepare the receptor, and all water molecules greater than 5 angstroms around the protein were removed. The potential allosteric sites of VHL and HIF-1α were predicted by AllositePro (Song et al., 2017). The prepared ligand was then flexibly docked into the predicted binding site of the receptor using the Glide XP mode with default parameters. Finally, several docking poses were obtained for the molecule, and the one with the best Glide score was chosen: The Glide score of ginsenoside Re docking with the VHL protein was -7.936, and the Glide score of ginsenoside Re docking with the HIF-1α protein was -7.391.

Allosteric Site Prediction

AllositePro is used to predict allosteric sites in proteins by combining pocket features with outcomes of a perturbation analysis. This feature-based model was trained with a high-quality benchmarking data set, ASBench (Huang et al., 2015), on the basis of a logistic regression method. Then, normal-mode analysis (NMA), an efficient means to study likely motion of interacting biomolecules, was used to evaluate the dynamic changes in proteins triggered by allosteric ligands. The score of the perturbation method (PNMA) was defined by the p-value determined by a Wilcoxon–Mann–Whitney test. In the final model, an allosteric site was identified by combining AllositePro and NMA data.

Luciferase Reporter Assay

HL-1 cells were seeded on 24-well plates (5×10^4 cells per well) and transfected with pRL-TK and HRE-luc reporter gene plasmids with or without ginsenoside Re (100 μM) treatment. Luciferase activity was measured as described (Ling et al., 2012; Clément et al., 2015). To normalize the results, Renilla luciferase was cotransfected. Luciferase activity was detected by a Dual-Luciferase® reporter assay system (Promega).

Real-Time PCR

Total RNA was extracted from the HL-1 cells using TRIzol reagent (TaKaRa, Kyoto, Japan) according to the manufacturer's instructions. Real-time PCR was performed in duplicate on a Light Cycler (Eppendorf, Hamburg, Germany) using SYBR green mix (S4438; Bio-Rad, Hercules, CA), and the expression of glucose transporter 1 (GLUT1) and regulated in development and DNA damage response 1 (REDD1) was normalized to that of GAPDH. The following primers were used:

GLUT1 sense primer: 5′-GTAGAGCGAGCTGGACG ACG-3′;

GLUT1 antisense primer: 5′-GGCCACGATGCTCAGAT AGG-3′;

REDD1 sense primer: 5′-CTTCGTCCTCGTCTCGAACT-3′;

REDD1 antisense primer: 5′-CCATCCAGGTATGAGG AGTCTTCC-3′;

GAPDH sense primer: 5′-ACTCCACTCACGGCAAA TTCA-3′; and

GAPDH antisense primer: 5′-GGCCTCACCCCATTTG ATG-3′.

Half-Life of HIF-1α Protein Degradation

HL-1 cells were seeded on 24-well plates and transfected with the HA-HIF-1α plasmid (100 ng/well). After culturing for 8 h, the cells were treated with ginsenoside Re (100 μM) or DMSO. Once the cells reached 70% confluence on the plates, cycloheximide (diluted 1:10,000) was added to the plates. Then, the cells were harvested every 2 h until 10 h after cycloheximide treatment. The level of HIF-1α protein was detected by Western blotting and quantified by ImageJ software. The HIF-1α protein half-life was calculated by linear regression analysis.

Immunoprecipitation and Immunoblotting

Lipofectamine 2000 (Invitrogen, USA) was used for transfection. Forty-eight hours after transfection, the HL-1 cells were harvested and lysed in 1 mL of lysis buffer (1% NP40, 10% glycerol, 135 mM NaCl, 20 mM Tris, 40 μl of 50× cocktail, 40 μl of 50×Na_3VO_4, 40 μl of 50×NaF, 40 μl of 50×Na_2PO_4, and 0.8 Ml of sterile water, pH 7.4). To determine the interaction between VHL and HIF-1α, immunoprecipitation was performed with 2 μg of agarose-conjugated rabbit anti-HIF-1α antibody (Cell Signaling). The proteins in both the total cell lysates and the immunoprecipitates were measured by immunoblotting in the presence of antibodies against VHL (diluted 1:1,000) and HIF-1α (diluted 1:1,000) (Ling et al., 2012).

Ubiquitination Assay

To detect exogenously induced ubiquitylation of HIF-1α, the HL-1 cells were transfected with His-Ub and HA-HIF-1α. The cells were treated with or without ginsenoside Re (100 μM), and then, the proteasome inhibitor MG132 (20 μM; Sigma) was added to the medium for 6 h before cell harvesting. Cell lysates were immunoprecipitated with the HA antibody (2 μg, #3724S, Cell Signaling Technology), followed by immunoblotting with anti-His antibody (diluted 1:1000, PM020-8, MBL).

Statistical Analyses

The data, represented as the mean ± standard error of the mean (SEM), were analyzed by one-way ANOVA. Post hoc analyses included the Student–Newman–Keuls method and Dunnett's test. Probability values <0.05 were considered significant. We used two-way analysis of variance with unequal variances to account for 2 factors and their interactions. Bonferroni's adjustment was used for multiple comparisons. Statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA).

RESULTS

Ginsenoside Re Increased Cell Viability and ATP Levels in HL-1 Cells After H/R Treatment

Compared with the control group, H/R treatment reduced HL-1 cell viability, while ginsenoside Re treatment increased cell viability following H/R injury (**Figure 1A**). To validate the role of ginsenoside Re treatment on the energy metabolism of the

cardiomyocytes, ATP levels were measured by enzymatic assays. A significant decrease in ATP level was observed after H/R induction, but the ATP level increased after ginsenoside Re treatment (**Figure 1B**).

Ginsenoside Re Treatment Attenuated Myocardial H/R Injury *via* the HIF-1 Signaling Pathway

A network pharmacology prediction analysis was performed to explore the potential molecular mechanism of ginsenoside Re action. The HIF-1 signaling pathway was predicted to be one of the most important pathways involved in the regulation of H/R (**Figures 2A, B**). To validate the signaling pathway predicted by the network pharmacology analysis, a luciferase reporter assay was performed. Compared with the HRE activity of the H/R group, treatment with ginsenoside Re increased the activity of HRE (**Figure 2C**). Inhibiting the HIF-1 signaling pathway by silencing *HIF-1α* attenuated the effect of ginsenoside Re on HL-1 cell viability, and the effect was reestablished after transfection with the HIF-1α-expressing plasmid (**Figure 2D**).

Ginsenoside Re Treatment Inhibits the Ubiquitination of HIF-1α

Docking and allosteric site analyses were performed to analyze the binding site where ginsenoside Re interacts with HIF-1 to initiate the signalling pathway, and the results showed that ginsenoside Re was incorporated into the binding interface of HIF-1α and the VHL protein, preventing VHL from binding HIF-1α and inhibiting the ubiquitination of HIF-1α (**Figures 3A–D**).

To validate the results of the bioinformatic analysis, real-time PCR and ubiquitination and immunoprecipitation assays were performed. Compared with the HIF-1α mRNA level in the H/R group, ginsenoside Re treatment did not change the level of HIF-1α mRNA (**Figure 4A**); however, following ginsenoside Re treatment, the level of HIF-1α increased (**Figure 4B**) and that of HIF-1α[Ub]n decreased (**Figure 4C**). The immunoprecipitation results showed that the quantity of HIF-1α binding to VHL decreased following ginsenoside Re treatment (**Figure 4D**).

Ginsenoside Re Treatment Increases the Expression of HIF-1α Target Genes

GLUT1 and REDD1 are the target genes of HIF-1α, and they are associated with energy metabolism and cell apoptosis, respectively. Real-time PCR was performed to determine the expression of the GLUT1 and REDD1 mRNA levels. Compared with their expression in the H/R group, ginsenoside Re treatment increased the expression of GLUT1 and REDD1 (**Figures 5A, B**).

DISCUSSION

In the present study, we demonstrated that ginsenoside Re treatment improved HL-1 cell viability and ATP levels following H/R. In addition, inhibiting the HIF-1 signaling pathway by silencing *HIF-1α* counteracted the effect of ginsenoside Re on HL-1 cell viability. Furthermore, the results of the bioinformatics analysis demonstrated that ginsenoside Re acted upon the binding interface between HIF-1α and VHL protein, preventing VHL from binding HIF-1α, thus inhibiting the ubiquitination of HIF-1α. To validate the results of the bioinformatic analysis, real-time PCR and ubiquitination and immunoprecipitation assays were performed. Compared with the level of HIF-1α mRNA in the the H/R group, ginsenoside Re treatment did not change the level of HIF-1α mRNA; however, following ginsenoside Re treatment, the level of HIF-1α increased and that of HIF-1α[Ub]n decreased. The results from the immunoprecipitation showed that the quantity of HIF-1α binding to VHL decreased following ginsenoside Re treatment.

HIF-1, composed of the HIF-1α functional subunit and the HIF-1β constitutional subunit (Luo et al., 2019), plays an important role in sensing and adapting to changes in oxygen concentration and is associated with cellular energy metabolism and apoptosis (Soni and Padwad, 2017). HIF-1 is an important target for diseases related to oxygen deprivation (Albadari et al., 2019). Some HIF-1α-based examples including antioxidation (iNOS), erythropoiesis (EPO) and angiogenesis (VEGF, ENG, LEP), counteract ischemia, hypoxia and promote angiogenesis (Jain et al., 2018). Previous studies demonstrated that HIF-1

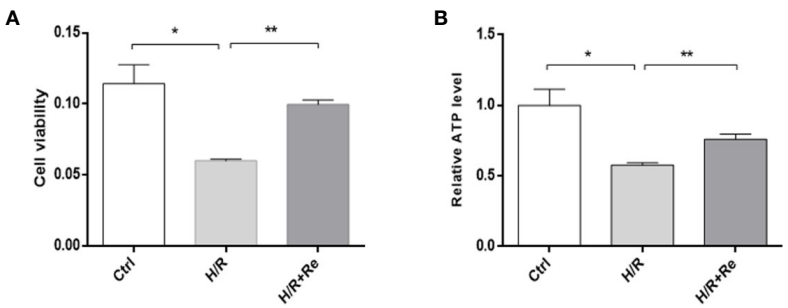

FIGURE 1 | HL-1 cells viability and ATP level after H/R. **(A)** HL-1 cells viability (n=5). **(B)** ATP levels (n = 5). All data are shown as mean ± SEM, *$P < 0.05$, **$P < 0.01$. Ctrl, control; H/R, hypoxia reoxygenation; RE, ginsenoside Re; ATP, adenosine triphosphate.

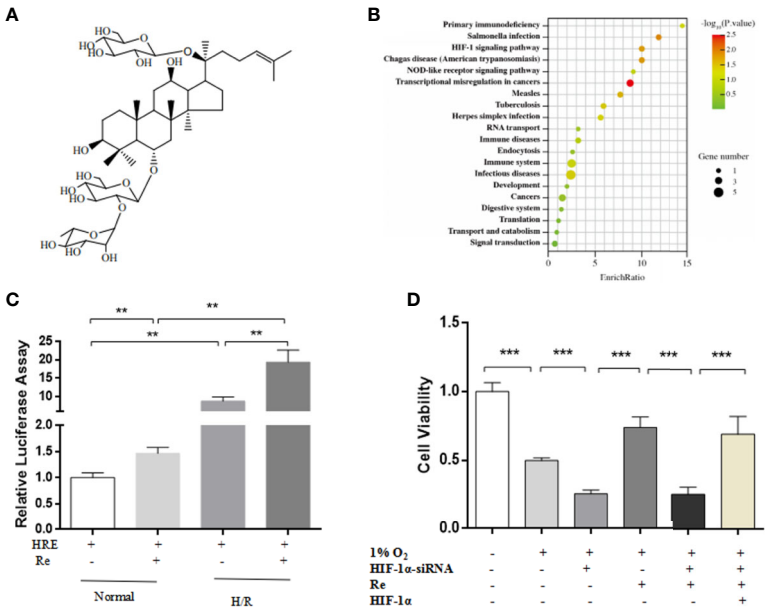

FIGURE 2 | HIF Signaling pathway was predicted by notework pharmacology and vadaliated by luciferase assay and gene siliencing. **(A)** Molecule structure of ginsenoside Re. **(B)** Signaling pathway predictd by network pharmacology. **(C)** Expression of HIF-1α signaling pathway was examined by relative luciferase assay (n = 5). **(D)** HL-1 cell viability after silencing HIF-1α (n = 5). All data are shown as mean ± SEM, $^{**}P < 0.01$, $^{***}P < 0.001$. H/R, hypoxia reoxygenation; RE, ginsenoside Re.

activation and upregulation of HIF-1α attenuate myocardial I/R injury (Yang et al., 2020). VHL, a tumor suppressor, functions as part of a ubiquitin ligase complex that recognizes HIF-1α as a substrate and is part of the oxygen-sensing mechanism of the cell (Vriend and Reiter, 2016). Under normoxic conditions, α-ketoglutarate-dependent prolyl hydroxylases (PHDs) catalyze the hydroxylation of proline residues in the oxygen-dependent degradation domains of HIF-1α, which are recognized by the VHL E3 ubiquitin ligase complex, leading to HIF-1α ubiquitination and subsequent degradation (LaGory and Giaccia, 2016). Inhibiting the combination of VHL and HIF-1α is an important step to decrease the ubiquitination of HIF-1α and improve myocardial cell viability. However, to date, no effective drugs have been developed for inhibiting the ubiquitination of HIF-1α (Zeng et al., 2017). The present study shows that ginsenoside Re treatment has a protective effect

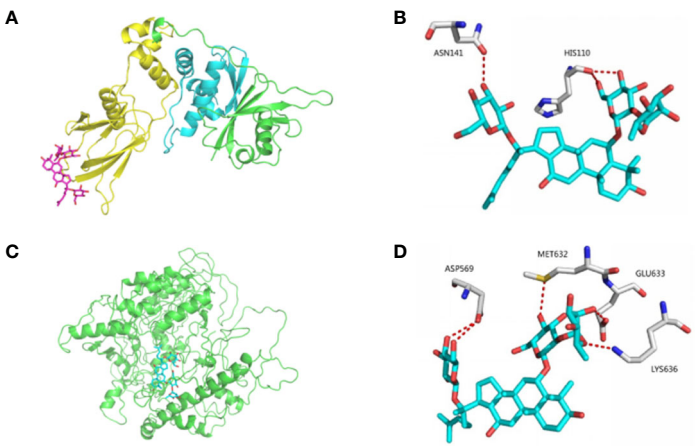

FIGURE 3 | The bindings among ginsenoside Re, VHL protein and HIF-1α protein. **(A, B)** The details of predicted binding mode of ginsenoside Re and VHL protein. The contact residues were shown and labeled by type and number. The red dotted line illustrated the hydrogen bond interaction. **(C, D)** The details of predicted binding mode of ginsenoside Re and HIF-1α protein. The contact residues were shown and labeled by type and number. The red dotted line illustrated the hydrogen bond interaction.

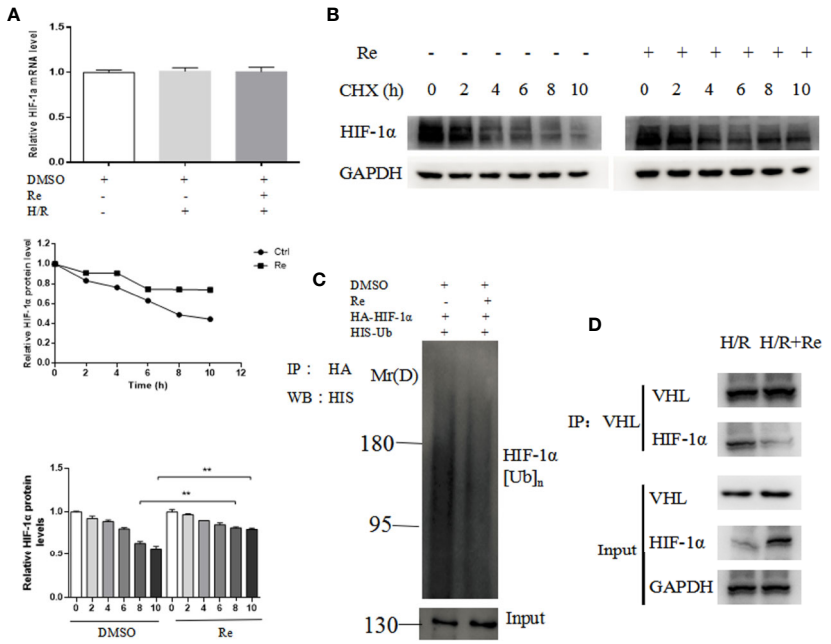

FIGURE 4 | Ubiquitination of HIF-1α following ginsenoside Re treatment. **(A)** HIF-1α mRNA level. **(B)** Half-life of HIF-1α protein. **(C)** HIF-1α ubiquitination following ginsenoside Re treatment. **(D)** Interaction between VHL and HIF-1α following ginsenoside Re treatment. All data are shown as mean ± SEM, $^{**}P < 0.01$. Ctrl, control; RE, ginsenoside Re; DMSO, Dimethyl sulfoxide.

against myocardial injury induced by H/R through the HIF-1 signaling pathway, and the results show that ginsenoside Re inhibited the combination of HIF-1α and VHL protein, thus inhibiting the ubiquitination of HIF-1α. ODDD domain in HIF-1α mediates its binding with VHL (Warren et al., 2019). It is important to determinate the specific site in this domain for ginsenoside Re binding.

Glucose transporters (Gluts) are involved in the transmembrane facilitated diffusion of glucose (Lu et al., 2019). Among them, GLUT1 is a basal glucose transporter that is widely expressed in cells and tissues (Mueckler and Thorens, 2013). In particular, GLUT1 is critical for the constant transport of glucose into erythrocytes through facilitated diffusion (Nagao et al., 2019). In our study, ginsenoside Re treatment increased the mRNA level of GLUT1, suggesting that ginsenoside Re improves energy metabolism in cardiomyocytes following H/R. REDD1 is an essential regulator of cell growth, and a low level of REDD1 expression is associated with cell apoptosis (Sun and Yue, 2019). Our study showed that ginsenoside Re treatment significantly increased the mRNA level of REDD1, indicating that the effects of ginsenoside Re on cardiomyocyte H/R injury are related to the inhibition of cell apoptosis.

GLUT1 is related to glucose transport, which is a part of energy metabolism. AMPK is a regulator of energy metabolism in the heart and is activated by falling energy status that boosts ATP production by stimulating glucose uptake and glycolysis (Cisternas et al., 2019). In our study, ginsenoside Re treatment increased the level of ATP and the mRNA level of GLUT1 following H/R, which implicated that the AMPK signaling pathway was involved in the regulation of ginsenoside Re on H/R. REDD1 regulates mTOR-dependent phosphorylation following AMPK activation, energy stress induces expression of REDD1, inhibiting mTOR activity, inhibits protein synthesis and decreases cellular growth (Seong et al., 2019). In the present study, ginsenoside Re treatment significantly increased the mRNA level of REDD1, which indicates that the effects of ginsenoside Re on cardiomyocytes H/R injury are mTOR signaling pathway-related. BNIP3, which is a pro-apoptotic protein and a member of Bcl-2 family, is the downstream of HIF-1 signal pathway (Cai et al., 2017). HIF-1α/BNIP3 signaling pathway-induced-autophagy plays protective role during myocardial ischemia-reperfusion injury (Zhu et al., 2020). HIF-1α/BNIP3 signaling pathway will

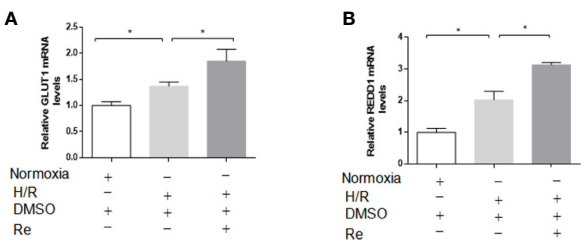

FIGURE 5 | Effects of ginsenoside Re on gene targets of HIF-1α. **(A, B)** mRNA levels of GLUT and REDD1 were measured by qPCR (n=5). All data are shown as mean ± SEM, $^{*}P < 0.05$. H/R, hypoxia reoxygenation; RE, ginsenoside Re; DMSO, Dimethyl sulfoxide.

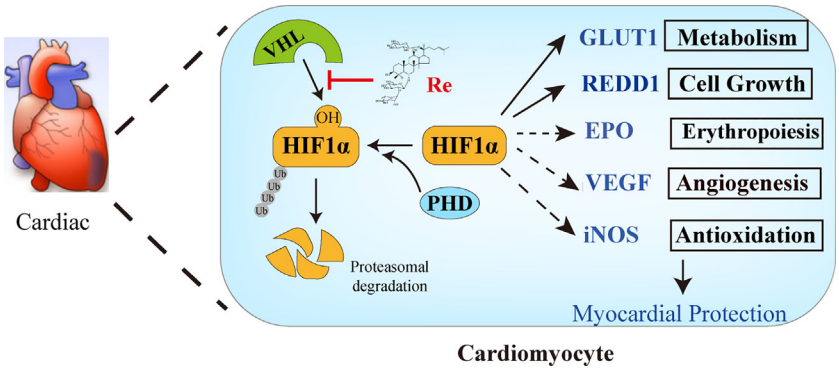

FIGURE 6 | The possible mechanism of ginsenoside Re attenuating myocardial injury induced by H/R. Ginsenoside Re inhibited the ubiquitination of HIF-1α *via* inhibiting the binding between VHL and HIF-1α. In addition, the target genes of HIF-1α, such as GLUT1 and REDD1, increased following ginsenoside Re treatment.

be explored in our next study. In the future, the underlying mechanisms will be further explored. The changes of AMPK/mTOR/HIF-1/BNIP3 signal pathways will be further clarified and the specific sites for Re binding domain will be identified in the next experiment.

Taken together, our present work has demonstrated that ginsenoside Re attenuated the myocardial injury induced by H/R. The possible mechanism is associated with the localization of ginsenoside Re at the binding interface between HIF-1α and VHL protein, preventing VHL from binding HIF-1α, thereby inhibiting the ubiquitination of HIF-1α (**Figures 6**).

DATA AVAILABILITY STATEMENT

The datasets analyzed in this article are not publicly available because further study will be done based on parts of these datasets as we mentioned in this paper. Requests to access the datasets should be directed to C729@bucm.edu.cn.

AUTHOR CONTRIBUTIONS

DS and YXL conceived the study. HS performed the experiment with support from SKL, DZ, JL, YHL, and CL. YL performed network pharmacology analysis. RD and GZ analyzed and interpreted the results. SL, ZL, XG, XJ, and FX provided intellectual contribution. HS wrote the manuscript under the guidance of DS, YXL, and SKL. DS, YXL, SKL, HQ, and YZ revised the manuscript and gave final approval of the submitted manuscript. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Efficacy of Intravenous Tranexamic Acid in Reducing Perioperative Blood Loss and Blood Product Transfusion Requirements in Patients Undergoing Multilevel Thoracic and Lumbar Spinal Surgeries: A Retrospective Study

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Introduction: Acute perioperative blood loss is a common and potentially major complication of multilevel spinal surgery, usually worsened by the number of levels fused and of osteotomies performed. Pharmacological approaches to blood conservation during spinal surgery include the use of intravenous tranexamic acid (TXA), an anti-fibrinolytic that has been widely used to reduce blood loss in cardiac and orthopedic surgery. The primary objective of this study was to assess the efficacy of intraoperative TXA in reducing estimated blood loss (EBL) and red blood cell (RBC) transfusion requirements in patients undergoing multilevel spinal fusion.

Materials and Methods: This a single-center, retrospective study of subjects who underwent multilevel (≥7) spinal fusion surgery who received (TXA group) or did not receive (control group) IV TXA at The Ohio State University Wexner Medical Center between January 1st, 2016 and November 30th, 2018. Patient demographics, EBL, TXA doses, blood product requirements and postoperative complications were recorded.

Results: A total of 76 adult subjects were included, of whom 34 received TXA during surgery (TXA group). The mean fusion length was 12 levels. The mean total loading, maintenance surgery and total dose of IV TXA was 1.5, 2.1 mg per kilo (mg/kg) per hour and 33.8 mg/kg, respectively. The mean EBL in the control was higher than the TXA group, 3,594.1 [2,689.7, 4,298.5] vs. 2,184.2 [1,290.2, 3,078.3] ml. Among all subjects, the mean number of intraoperative RBC and FFP units transfused was significantly higher in the control than in the TXA group. The total mean number of RBC and FFP units transfused in

Abbreviations: ABT, allogeneic blood transfusions; ASA, American Society of Anesthesiology; CONSORT, Consolidated Standards of Reporting Trials; CI, confidence interval; DVT, deep vein thrombosis; EBL, estimated blood loss; ICU, intensive care unit; IV, intravenous; RBCs, red blood cells; FFP, fresh frozen plasma; TXA, tranexamic acid; PACU, post-anesthesia care unit; PE, pulmonary embolism.

the control group was 8.1 [6.6, 9.7] and 7.7 [6.1, 9.4] compared with 5.1 [3.4, 6.8] and 4.6 [2.8, 6.4], respectively. There were no statistically significant differences in *postoperative* blood product transfusion rates between both groups. Additionally, there were no significant differences in the incidence of 30-days postoperative complications between both groups.

Conclusion: Our results suggest that the prophylactic use of TXA may reduce intraoperative EBL and RBC unit transfusion requirements in patients undergoing multilevel spinal fusion procedures ≥7 levels.

Keywords: neurosurgery, spinal surgery, blood loss, tranexamic acid, spinal fusion, blood transfusion

INTRODUCTION

Multilevel spinal fusion surgery is among the most common procedures (ranked fifth in 2017) performed on inpatients in the United States (Healthcare Cost and Utilization Project (HCUP), 2017; Goobie et al., 2018; Verma et al., 2010). Acute perioperative blood loss is a common and potentially major complication of multilevel spinal surgery, usually worsened by the number of levels fused and of osteotomies performed (Lin et al., 2018; Lu et al., 2018; Shakeri et al., 2018). For cases involving more levels, the larger operative wound and area of bleeding surfaces exposed have been associated with increased blood loss (Nagabhushan et al., 2018).

There are several patient factors that contribute to the significant intraoperative blood loss in spinal fusion surgery including, but not limited to: height of the patient, length of surgical field exposure and severity or type of spine deformity (Verma et al., 2010). Additionally, there are surgical factors that include: length of surgery, type of procedure, combined approaches (anterior and posterior), number of levels fused, number of anchors placed, intraoperative maintenance of the mean arterial pressure, utilization of blood salvage techniques, history of coagulopathy and the use of anti-fibrinolytic drugs (Verma et al., 2010). Also, the prone position might lead to hemodynamic challenges during surgery by restricting the blood flow of the inferior vena cava and consequently distending the paravertebral and epidural veins, which contributes to increased bleeding (DePasse et al., 2015). In order to minimize this, the use of the Jackson table has been recommended due to its capacity to reduce intra-abdominal pressure (Jackson, 1992; Mathai et al., 2012).

The prevention of blood loss is crucial to spinal surgery since massive blood loss can contribute to coagulopathy and disseminated intravascular coagulation, increasing the risk of postoperative hematoma and neurological deficits (Hu, 2004; Elgafy et al., 2010).

Traumatic hemorrhage, as well as profuse intraoperative bleeding, promotes massive release of tissue plasminogen activator (tPA) inducing hyperfibrinolysis (Kashuk et al., 2010; Chapman et al., 2016). Plasminogen, produced by the liver, and tPA bind to C-terminal lysine residues on fibrin leading to localized plasmin formation and fibrin cleavage (Chapman et al., 2016). Tranexamic Acid (TXA) is an anti-fibrinolytic that has been widely used to prevent massive blood loss in cardiac and orthopedic surgery, with an increased use in spinal surgeries. (Verma et al., 2010; Hunt, 2015; Yoo et al., 2019). TXA inhibits the capacity of plasminogen and plasmin to bind to fibrin, hence preserving blood clots from plasmin-mediated lysis (Verma et al., 2010; Hunt, 2015). TXA, a synthetic derivative of lysine, exerts its antifibrinolytic activity by reversibly and competitively binding to lysine-binding sites on the structural proteins of plasmin, plasminogen, and as well as tPA, this consequently inhibits the degradation of fibrin molecules (McCormack, 2012). Each plasminogen molecule has up to five binding sites for TXA, with one site of high affinity (McCormack, 2012). Data from animal models of traumatic injuries also suggests a delayed release of urokinase plasminogen activator (uPA) (Hijazi et al., 2015). Physiologically, TXA catalyzes the conversion of plasminogen to active plasmin, which induces controlled anticoagulation and clot breakdown (Hijazi et al., 2015). There is evidence showing that TXA has pharmacological actions other than inhibition of fibrinolysis (Couturier and Grassin-Delyle, 2014), such as clot stabilization (Dai et al., 2011), improvement of platelet function (Blake et al., 2006), inhibition of apoptosis (Hsia et al., 2010), and prevention of pro-inflammatory cytokine production (Jimenez et al., 2007). However, its safety and efficacy profiles are not well known in spinal surgery (Lin et al., 2018; Lu et al., 2018; Luo et al., 2018; Nagabhushan et al., 2018; Shakeri et al., 2018; Xue et al., 2018; Karimi et al., 2019).

The literature describes different volume, timing and route of TXA administration for spinal procedures. Intravenous administration is the most Common, ranging from 10–20 mg per kilogram (mg/kg) as a loading dose and 1–10 mg per kilogram per hour (mg/kg/h) as a maintenance dose (Walterscheid et al., 2017). Similar to its use in other surgical procedures, the most common side effects related to TXA are seizures, acute kidney injury, liver injury, and mainly thromboembolic events (myocardial infarction, stroke, deep vein thrombosis and pulmonary embolism), particularly in elderly patients (Neilipovitz et al., 2001; Verma et al., 2010; Colomina et al., 2017; Grassin-Delyle et al., 2018; Nagabhushan et al., 2018). The efficacy of its use has been assessed in spinal surgery and the results are inconclusive due to the complexity and diversity of the dosing regimen (Neilipovitz et al., 2001; Wong et al., 2008; Verma et al., 2010; Farrokhi et al., 2011; Colomina et al., 2017; Johnson et al., 2017; Carabini et al., 2018; Grassin-Delyle et al., 2018; Lin et al., 2018; Lu et al., 2018; Nagabhushan et al., 2018; Karimi et al., 2019). After the consideration of intraoperative blood loss and the incidence of autologous transfusion, the benefit of using TXA might outweigh the undesirable side effects. However, although it is widely used, it is not currently considered part of standard of care in spinal surgeries (Verma et al., 2010; Walterscheid et al., 2017).

The efficacy profile of TXA in spinal procedures remains unclear and should be investigated. Therefore, we designed a retrospective study with the hypothesis that patients undergoing primary or revision spinal fusion surgery (≥7 levels) who received intravenous (IV) TXA would have a significant reduction in intraoperative estimated blood loss (EBL) and red blood cell (RBC) transfusions compared with patients who did not receive IV TXA. The primary objective of the study was to assess the efficacy of intraoperative TXA in reducing EBL and RBC transfusions in patients undergoing multilevel spinal fusion.

MATERIALS AND METHODS

Research Design

We conducted a single-center, retrospective study that reviewed electronic medical records from subjects who underwent multilevel (≥7) spinal fusion surgery and received (TXA group) or did not receive (control group) IV TXA at The Ohio State University Wexner Medical Center between January 1st, 2016 and November 30th, 2018. The decision of dosing TXA during surgery was at the surgeon's discretion. After obtaining the approval of our Institutional Review Board (Office of Responsible Research Practices, The Ohio State University), we accessed electronic medical records in order to assess eligibility and collect perioperative information from 404 subjects.

Participants

Subjects 18 years and older that underwent ≥7 levels spinal fusion surgery (primary or revision) using a posterior midline approach and received loading and maintenance dose TXA between January 1st, 2016 and November 30th, 2018 at The Ohio State University Wexner Medical Center, Department of Neurosurgery, were included in the study. Prisoners, pregnant women, history of congenital coagulation disorders, chronic kidney disease (baseline serum creatinine >1.36 mg per deciliter [mg/dl]), chronic liver disease, concomitant use of coagulation-altering medications, or undergoing active treatment for malignancies were excluded from the study.

Study Procedures

The following information was collected for analysis purposes: demographics (age, gender, race, height, weight, BMI), the American Society of Anesthesiology (ASA) physical status, perioperative coagulation and hematologic parameters, surgical variables (number of levels fused, length of surgery and of anesthesia, previous spinal surgery, spinal surgeon, type of spinal pathology, type of surgical technique, EBL, units, volume and type of blood transfusions administered (considering that the threshold for blood products transfusion was hemoglobin ≤7 mg/dl), total dose of IV TXA administration, length of post-anesthesia care unit (PACU) and hospital stay. In addition, type and units of blood product transfusions administered during the first 24 h after the end of surgery and any complications diagnosed in a 30-days period after surgery were also collected. Intraoperative transfusions were performed in accordance with our institutional transfusion protocol ("Transfusion Therapy: Indications for Ordering"). If the patient was hemodynamically stable, intraoperative blood transfusion was considered at a hemoglobin threshold of <8 gm/dl, if intraoperative bleeding is not expected to be life-threaten. In addition, the decision to transfuse blood products during surgery was made by the anesthesiology care provider following our institutional transfusion protocol. Consequently, as part of our institutional transfusion protocol, platelets can be ordered for pooled (4 or 6 units) prior to surgery and if more units are required, single units are dispensed and all blood products used at our institution are leukoreduced. In addition, the intraoperative estimation of EBL was made according to the standard procedure as the difference between the total surgical suctioned volume and the total amount of irrigation used during the case. The hemoglobin concentration was measured in all patients preoperatively. All gauzes and lap sponges were wringed into the field, circulated through the suction device, and accounted for by EBL. Postoperative EBL was estimated by postoperative drain output.

Outcomes

The primary outcomes were incidence and quantity of intraoperative EBL and RBC transfusions. Our secondary outcomes were the incidence and quantity of other perioperative blood product transfusion requirements, surgical variables (length of surgery, anesthesia, PACU, intensive care unit (ICU) and hospitalization stay) the incidence of 30-days postoperative complications in both groups.

Statistical Methods

Continuous patient demographic and clinical/surgical variables were summarized as means (±standard deviations) and categorical variables were summarized as frequencies (percentages). Student's t-tests or Mann-Whitney U tests were used to test demographic differences between study groups for continuous variables where appropriate. Chi-square or Fisher's exact tests were used to test for differences in categorical variables between study groups where appropriate. Linear regression models were fit for each of the outcomes in the primary aim adjusting for potential confounders and effect modifiers, including number of spinal levels, cell saver use and number of surgeons variable. The final models for RBC and FFP included main effects for study group and number of spine levels. The final model for EBL also included an interaction term between study group and number of spine levels. All models were checked to verify that statistical assumptions of normality of residuals and equal variance were not violated. Hypothesis testing comparing outcomes between study groups was administered based on the final model estimates for each outcome and conducted at a two-sided alpha of 0.05. All statistical analyses were performed using SAS/STAT software version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

A total of 404 charts were initially reviewed. Data from 328 subjects were not eligible based on inclusion and exclusion criteria; the main reasons for exclusion were cervical spinal surgeries, fewer than seven levels fused and minimally invasive

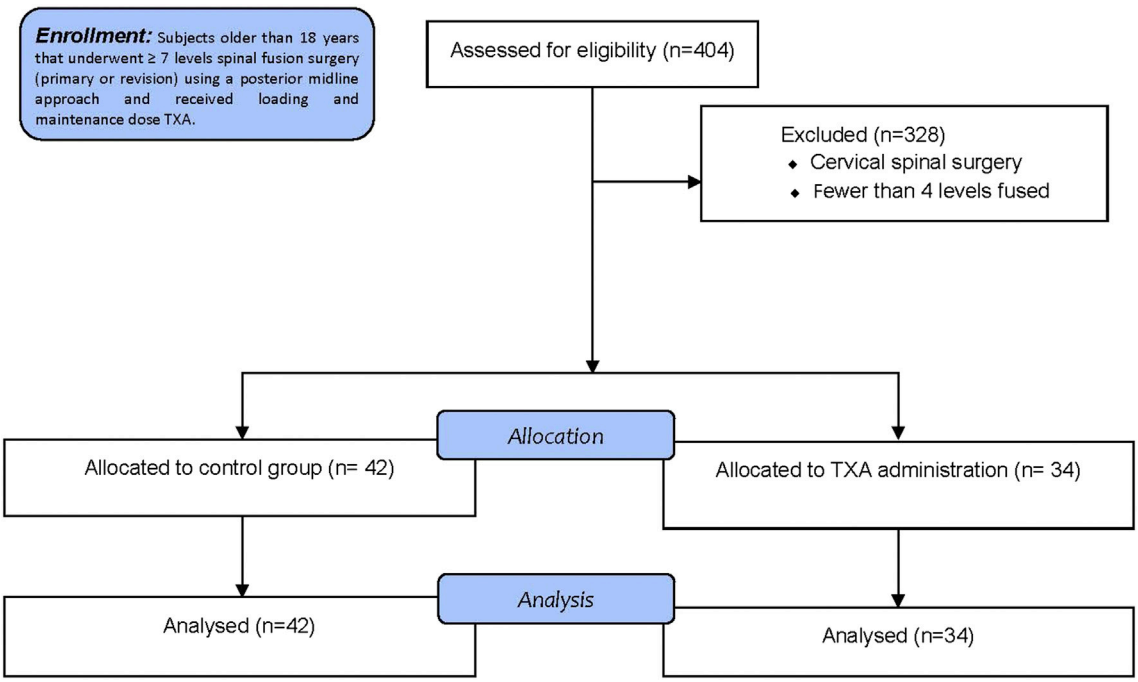

FIGURE 1 | Retrospective analysis consort flow diagram. N, number; TXA, tranexamic acid.

procedures. Therefore, this study included 76 subjects for data analysis, of whom 42 (55.3%) did not receive IV TXA (control group) and 34 (44.7%) received IV TXA (TXA group) during surgery. The flow-diagram of this retrospective observational trial according to the Consolidated Standards of Reporting Trials (CONSORT) is shown in **Figure 1** (Begg et al., 1996).

A summary of demographic and perioperative variables is listed in **Table 1**. The mean age was 59.3 (±12.0) years in the control group and 58 (±12.0) years in the TXA group. The mean total loading dose, dose rate for maintenance and total dose administered during maintenance of IV TXA administration was 1.5 (±0.9) mg, 2.1 (±1.3) mg/kg/h and 33.8 (±20.3) mg/kg, respectively. There was not a statistically significant difference in demographic and clinical characteristics except for length of anesthesia and surgery. Subjects in the TXA group had a significantly shorter mean length of anesthesia (9.8 ± 1.8 vs. 10.9 ± 2.3 h; p-value = 0.0296) and surgery (7.2 ± 1.6 vs. 8.3 ± 2.3 h; p-value = 0.0227).

The mean EBL in the control was higher than the TXA group, 3,494.1 [2,689.7, 4,298.5] vs. 2,184.2 [1,290.2, 3,078.3] ml (95% confidence interval [CI]; p-value = 0.0332) (**Table 2**). Among all subjects, the mean number of intraoperative RBC and FFP units transfused was significantly higher (95% CI; p-value < 0.05) in the control group than in the TXA group. The total mean number of RBC and FFP units given to subjects in the control group was 8.1 [6.6, 9.7] and 7.7 [6.1, 9.4] compared with 5.1 [3.4, 6.8] and 4.6 [2.8, 6.4], respectively (**Table 2**).

A multivariate model predicting EBL was fit including main effects for study group, number of spine levels and the interaction between the two variables. EBL increased at a greater rate for the control group compared to the TXA group as number of spine levels increased (interaction term p-value = 0.0386) (**Table 3**; **Figure 2**). Additionally, a multivariate model predicting RBC transfusion was fit including main effects for study group and number of spine levels. RBC transfusion was significantly higher on average for the control group compared to the TXA group (p-value = 0.0123). There was also a significant increase in blood loss as number of spine levels increased (p-value = 0.0001). An interaction term between study group and the number of spine levels was not included in the final multivariable RBC model as it was not statistically significant (p-value = 0.1708) (**Table 3**; **Figure 3**). Ultimately, a multivariate model predicting FFP transfusion was fit including main effects for study group and number of spine levels. FFP was significantly higher for the control group compared to the TXA group (p-value = 0.0143) after adjusting for number of spine levels. An interaction term between study group and number of spine levels was not included in the final multivariable FFP administration model as it was not statistically significant (p-value = 0.1697) (**Table 3**; **Figure 4**).

Subsequently, there was no statistical difference in *postoperative* blood product transfusion rates between both groups. As a final point, there was no significant difference in the incidence of 30-days postoperative complications between both groups (**Table 4**).

DISCUSSION

In the present retrospective study, 76 subjects underwent multilevel (≥7 levels) spinal fusion and 34 of them received

TABLE 1 | Demographic and perioperative variables.

Demographic and perioperative variables	Overall (n = 76)	Control (n = 42)	TXA (n = 34)	*p*-value
Age, years, mean (SD)	58.8 (11.9)	59.3 (12.0)	58 (12.0)	0.6383
Sex, male, n (%)	37 (48.7)	17 (40.5)	20 (58.8)	0.1116
Sex, female, n (%)	39 (51.3)	25 (59.5)	14 (41.2)	
Height, cm, mean (SD)	168.9 (11.0)	168.7 (11.4)	169.1 (10.6)	0.8695
Weight, kg, mean (SD)	85.4 (23.8)	85 (25.5)	85.8 (21.7)	0.8713
BMI, kg/m^2, mean (SD)	29.7 (7.0)	29.6 (7.7)	29.8 (6.2)	0.9074
ASA classification, I/II/III/IV, n	0/19/55/2	0/8/33/1	0/11/22/1	0.4233
Number of fusion levels, mean (SD)	12.3 (3.4)	12.5 (3.5)	12.0 (10.8)	0.5289
Length of anesthesia, hours, mean (SD)	10.4 (2.1)	10.9 (2.3)	9.8 (1.8)	**0.0296**
Length of surgery, hours, mean (SD)	7.8 (2.1)	8.3 (2.3)	7.2 (1.6)	**0.0227**
ICU admission, n (%)	71 (93.4)	38 (90.5)	33 (97.1)	0.3725
Cell saver used, n, (%)	8 (10.5)	6 (14.3)	2 (5.9)	0.2853
ICU length of stay, days, mean (SD)	3.1 (2.9)	3.1 (3.2)	3.1 (2.5)	0.9713
Length of hospital stay, days, mean (SD)	8.8 (3.6)	8.8 (3.1)	8.7 (4.2)	0.8478
Surgeon A, n (%)	60 (78.9%)	31 (73.8)	29 (85.3)	0.22
Previous spine surgery	35 (46.1)	21 (50)	14 (41.18)	0.4429
Pathology type				
Fracture	6 (7.9)	4 (9.52)	2 (5.88)	0.6858
Tumor	0	0	0	NA
Degenerative	26 (34.2)	12 (28.6)	14 (41.2)	0.2494
Deformity	52 (68.4)	29 (69.1)	23 (67.7)	0.8961
Spinal stenosis	33 (43.4)	21 (50)	12 (35.3)	0.1984
Foraminal stenosis	15 (19.7)	6 (14.29)	9 (26.47)	0.1845
Type of surgical technique				
Complex fusion	76 (100)	42 (55.0)	34 (45.0)	NA
Decompression	50 (65,8)	28 (66.67)	22 (64.71)	0.8578
Osteotomy	76 (100)	42 (55.0)	34 (45.0)	NA
Baseline hematologic and coagulation tests				
Hb, g/dL, mean (SD)	13.2 (1.9)	13.0 (1.7)	13.5 (2.0)	0.314
Ht, %, mean (SD)	40.2 (5.2)	39.6 (5.0)	40.8 (5.4)	0.3173
Platelets, 10^3/L, mean (SD)	247.7 (73.7)	244.3 (68.8)	251.8 (80.1)	0.6638
PT, s, mean (SD)	13.5 (1.8)	13.3 (0.9)	13.8 (2.5)	0.2864
PTT, s, mean (SD)	29.9 (4.2)	29.7 (3.9)	30.2 (4.6)	0.5477
INR, mean (SD)	1.1 (0.2)	1.0 (0.1)	1.1 (0.3)	0.2593
TXA total loading dose, g, overall, mean (SD)			1.5 (0.9)	
TXA dose rate, mg/kg/h, overall, mean (SD)			2.1 (1.3)	
TXA total dose, mg/kg, overall, mean (SD)			33.8 (20.3)	

N, number; %, percentage; cm, centimeter; SD, standard deviation; kg, kilogram; BMI, body mass index; m2, meter square; ICU, intensive care unit; ASA, American Society of Anesthesiology physical status classification; Hb, hemoglobin; g/dL, Gram per deciliter; L, liter; ht, hematocrit; PT, prothrombin time; s, seconds; PTT, Partial Thromboplastin Time; INR, international Normalized Ratio; TXA, tranexamic acid; g, Gram; mg/kg/hr, milligram per kilogram per hour; mg/Kg, milligram per kilogram.

TABLE 2 | Estimated blood loss and amount of intraoperative blood transfusion from univariate models.

Blood Products and EBL	Control (n = 42)	TXA (n = 34)	*p*-value
EBL, ml, overall, mean (95% CI)	3,494.07 (2,689.68, 4,298.46)	2,184.24 (1,290.21, 3,078.26)	**0.0332**
Blood product transfusions			
RBC, units, overall, mean (95% CI)	8.12 (6.57, 9.67)	5.09 (3.37, 6.81)	0.0110
FFP, units, overall, mean (95% CI)	7.71 (6.08, 9.35)	4.59 (2.77, 6.40)	0.0128

EBL, estimated blood loss; n, number; TXA, tranexamic acid; %, percentage; CI, confidence interval; RBC, red blood cells; FFP, fresh frozen plasma.

intraoperative loading and maintenance doses of TXA. Both groups were similar in the demographics and surgical procedure. The primary objective of the study was to assess the efficacy of intraoperative TXA in reducing EBL and RBC transfusions in patients undergoing multilevel (≥7 levels) spinal fusion. The results have shown significant difference in both groups; EBL during surgery and the number of intraoperative RBC transfused was reduced by 37% with the use of IV TXA. There was also a significant difference in the number of intraoperative RBC and FFP units transfused with the need of intraoperative transfusion being reduced by 37% and 40% in the TXA group compared to the control group (p-value = 0.0123 and 0.0143). A mean IV TXA loading and maintenance dose of 1.5 g and 2.1 mg/kg/h was well tolerated by subjects in the TXA group

TABLE 3 | Estimated blood loss and amount of intraoperative blood transfusion from multivariate models.

Blood Products and EBL	Control (n = 42)	TXA (n = 34)	*p*-value	Interaction with no. of spine levels
EBL,[a] mL, overall, mean (95% CI)	3,391.64 (2,666.94, 4,116.35)	2,217.01 (1,410.50, 3,023.54)	**0.0341**	**0.0386**
Blood product transfusions				
RBC,[b] units, overall, mean (95% CI)	7.98 (6.57, 9.38)	5.26 (3.70, 6.83)	0.0123	
FFP,[b] units, overall, mean (95% CI)	7.56 (6.09, 9.04)	4.78 (3.14, 6.42)	0.0143	

EBL, estimated blood loss; n, number; TXA, tranexamic acid; %, percentage; CI, confidence interval; RBC, red blood cells; FFP, fresh frozen plasma.
[a]*Adjusted of no. of spine levels and interaction of spine levels and study group.*
[b]*Adjusted for no. of spine levels.*

and there were no significant differences in the postoperative complications associated with its use. The results from this study will serve to direct the design and power analysis of a prospective double-blind randomized clinical trial assessing efficacy of using TXA administration in patients undergoing multilevel spinal fusion.

Complex multilevel spinal decompression, fusion and osteotomies have been associated with profuse perioperative blood loss and greater use of blood transfusions with subsequent increased risk of coagulation impairment, blood-borne infectious disease transmission, postoperative hematoma formation, shock and pulmonary edema (Johnson et al., 1989; Mirza et al., 2008; Naik et al., 2017). Studies describing the impact of perioperative TXA administration on blood loss and blood transfusion may vary among institutions. Choi et al. reviewed 132 consecutive patients (adults and children) undergoing multilevel (≥5 levels) posterior spinal instrumented fusion (Choi et al., 2017). TXA was administered in 67% of the patients (n = 89). For adult patients, an IV TXA loading dose of 10 mg/kg was used, followed by a continuous maintenance infusion dose of 1 mg/kg/h (Choi et al., 2017). In pediatrics (<18 years old), IV TXA 5 mg/kg/h. was used for maintenance following a 50 mg/kg loading dose (Choi et al., 2017). A significant reduction in EBL (841 vs. 1,396 ml; p = 0.002), intra- and postoperative transfusion volume (544 vs. 812 ml; p = 0.012 and 193 vs. 359 ml; p = 0.034, respectively) was reported in patients who received TXA when compared to patients in which TXA was not used (Choi et al., 2017). A multiple regression analysis demonstrated an association between TXA administration and decreased surgical bleeding (Choi et al., 2017).

Loading TXA doses are highly variable among published randomized clinical trials (RCTs) conducted in patients undergoing spinal surgeries, whereas infusion or maintenance doses seem to be more consistent. Moreover, reported intra- and postoperative outcomes may also vary. Shakeri et al. studied the efficacy of a single TXA dose of 15 mg/kg compared to placebo in 50 patients undergoing laminectomy (≥2 levels) with postero-lateral fusion. A significant reduction in mean total amount of bleeding (632.2 vs. 1,037 ml; p = 0.0001), RBC transfusion required (440 vs. 1,320 ml; p = 0.0001) and length of hospital stay (2.28 vs. 3.36 days, p = 0.001) was reported in patients who received TXA (Shakeri et al., 2018).

In a multicenter study, Colomina et al. randomized 95 patients undergoing posterior spinal surgery to receive either IV TXA (10 mg/kg infusion in 20 min before surgical incision followed by a 2 mg/kg/h until closure) or matched saline solution (Colomina et al., 2017). Patients undergoing thoracic or lumbar spinal fusions (>3 levels) were included in this study. Posterior and posterolateral techniques, spinal osteotomies, posterior lumbar interbody fusion (PLIF), transforaminal lumbar interbody fusion (TLIF) and instrumented procedures of at least four vertebrae (i.e. wires, pedicle screws) were some of the types of surgeries included (Colomina et al., 2017). The mean intraoperative and total blood loss was significantly reduced in patients receiving TXA when compared to placebo (p = 0.01) (Colomina et al., 2017). However, total number of RBC transfused was similar between groups (0.85 vs. 1.42 respectively, p = 0.06) (Colomina et al., 2017). Conversely, Carabini et al. reported a significant reduction in RBC transfused in patients receiving TXA (loading dose 10 mg/kg followed by 1 mg/kg/h maintenance infusion) for complex spinal fusions when compared to matched placebo (1,140 ml vs. 1,460 respectively, p = 0.034). Moreover, TXA use was also associated with significant decreased cell saver transfusion (256 vs. 490 ml respectively, p = 0.042) (Carabini et al., 2018).

A meta-analysis of 11 RCTs published in 2015 by Cheriyan et al. reported variability on TXA doses from 1 to 15 mg/kg among 644 patients undergoing spinal surgeries (Cheriyan et al., 2015). A significant reduction in intra- and postoperative blood loss was associated with TXA administration (Cheriyan et al.,

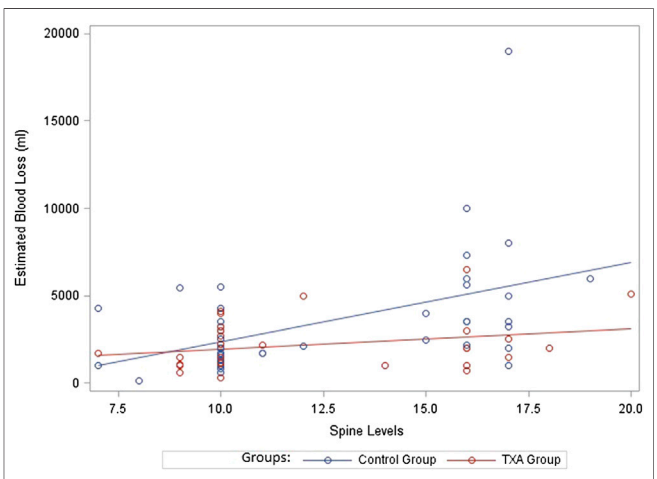

FIGURE 2 | Estimated blood loss. ml, milliliters; TXA, tranexamic acid.

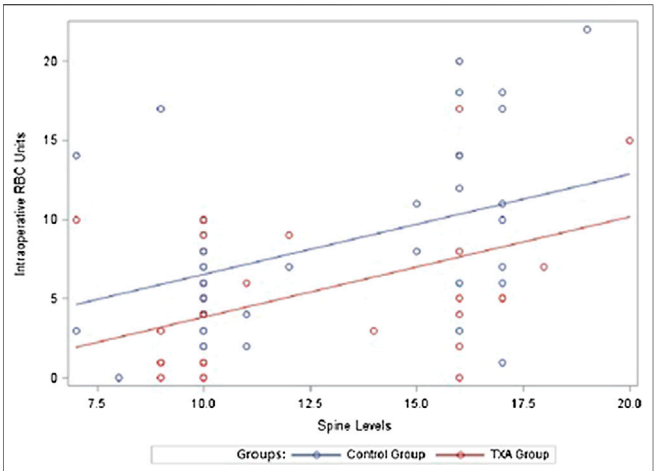

FIGURE 3 | Intraoperative transfused red blood cell units. RBC, red blood cells; TXA, tranexamic acid.

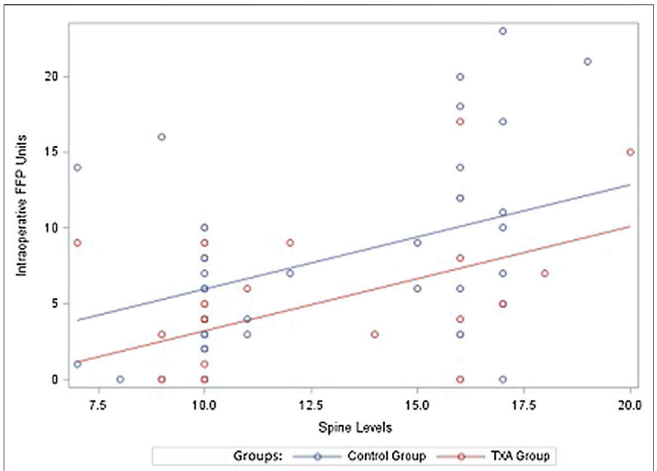

FIGURE 4 | Intraoperative transfused frozen fresh plasma units. FFP, fresh frozen plasma; TXA, tranexamic acid.

2015). Moreover, total perioperative blood loss was significantly lowered in patients receiving TXA when compared to placebo (p-value <0.0001) with no increase in the incidence of thromboembolic events among trials (Cheriyan et al., 2015).

Another systematic review and meta-analysis recently published in 2019 by Zhang et al. assessed the influence of TXA on blood loss and blood transfusion in multiple-level spine surgery from six RCT studies and five retrospective studies (Zhang et al., 2019). This study demonstrated that the administration of TXA can effectively decrease intraoperative blood loss and blood loss in spinal surgeries in contrast to the control group (p-value = 0.004) (Zhang et al., 2019). In addition, this study showed that the TXA group could maintain higher levels of postoperative hemoglobin when compared to the control group (p-value 0.009) (Zhang et al., 2019).

Goobie et al. studied the effect of higher TXA doses in 111 adolescent patients undergoing spinal surgery due to idiopathic scoliosis (Goobie et al., 2018). Patients were randomized into two groups: TXA (loading dose of 50 mg/kg after anesthesia induction followed by a maintenance dose of 10 mg/kg/h) or placebo (Goobie et al., 2018). The TXA group experienced a significantly lower amount of intraoperative bleeding (per spine level, rate per hour and total) when compared to placebo (p = 0.01, p < 0.001, and p = 0.02 respectively) (Goobie et al., 2018). Moreover, placebo was associated with a significant increase in postoperative bleeding when compared to the TXA group (645 ± 318 ml vs. 498 ± 228 respectively, p = 0.009) (Goobie et al., 2018). In addition, the relative risk of relevant blood loss (defined by authors as >20 ml/kg) was increased by 2.1 in patients receiving placebo with no thromboembolic events or seizures reported in any of the groups (Goobie et al., 2018). Nevertheless, in a series of 100 cases of patients undergoing complex spinal surgery and receiving a loading IV TXA dose of 50 mg/kg and a maintenance dose of 5 mg/kg/h, Lin et al. reported an incidence

TABLE 4 | Postoperative complications.

Post-operative complication (at 30 days)	Overall (N = 76)	Control (N = 42)	TXA (N = 34)	p-value
Incidence of complications, n, (%)	35 (46.05)	19 (45.24)	16 (47.06)	0.8742
Complications list, n, (%)				
Reoperations	5 (6.58)	3 (7.14)	2 (5.88)	>0.9999
Readmissions	11 (14.47)	4 (9.52)	7 (20.59)	0.1728
Wound infection	1 (1.32)	1 (2.38)	0 (0.00)	>0.9999
Pulmonary embolism	2 (2.63)	0 (0.00)	2 (5.88)	0.1968
Deep vein thrombosis	3 (3.95)	2 (4.76)	1 (2.94)	>0.9999
Seizures	1 (1.32)	1 (2)	0 (0.00)	>0.9999
Myocardial infarction	0 (0.00)	0 (0.00)	0 (0.00)	—
Stroke	0 (0.00)	0 (0.00)	0 (0.00)	—
Acute kidney injury	3 (3.95)	2 (4.76)	1 (2.94)	>0.9999
Hepatic dysfunction	0 (0.00)	0 (0.00)	0 (0.00)	—
Others	25 (32.89)	12 (28.57)	13 (38.24)	0.4634
Death	0 (0.00)	0 (0.00)	0 (0.00)	>0.9999

N, number; TXA, tranexamic acid; %, percentage.

of 3% of thromboembolic events potentially related to TXA administration (Goobie et al., 2018). One patient (n = 1) experienced pulmonary embolism (PE) whereas deep vein thrombosis (DVT) was diagnosed in two patients. No myocardial infraction, seizures, stroke or renal failure was reported (Goobie et al., 2018).

This retrospective study demonstrated a higher portion of PE with TXA (2 subjects (4.3%) vs. 0%, $p = 0.0487$, respectively). When analyzing thrombotic complications together (PE and DVT) there was no difference between TXA and control. We believe this is likely due to the relatively small size of this study.

An essential limitation of this observational study was its retrospective design and single-center population. Therefore, the high variability of the data limited the number of eligible subjects for data analysis. Secondly, due to the retrospective nature of this study, the doses of loading and maintenance dose were not standardized and were instead at the surgeon's discretion, leading to variability in dosing and possible influence over the results. Thirdly, as described by several studies with similar patient population, blood loss is also affected by other variables, such as the number and type of osteotomies performed (posterior column osteotomy, pedicle subtraction osteotomy or vertebral column resection) and the number and type of interbody fusions (posterior or transforaminal) performed (Johnson et al., 1989; Mirza et al., 2008; Naik et al., 2017). Fourthly, the number of subjects included in the data analysis for each group was not similar, resulting in 42 subjects in the control group and 34 in the TXA group. Lastly, this study was not powered to assess the hypothesis that the administration of IV TXA could decrease EBL and/or RBC units transfused.

CONCLUSION

The results from the statistical analysis of this study suggest that the prophylactic use of intraoperative IV TXA during multilevel spinal fusion surgery could reduce intraoperative EBL and RBC unit transfusion requirements. Due to associated limitations of the retrospective design of this study, our findings should be corroborated in a prospective randomized dose-response study for further evaluation of the efficacy of standardized doses of TXA administration in this patient population.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Office of Responsible Research Practices—The Ohio State University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Study conception and design: AU, AT, ME-V, JF-D, BM, AG, SV, SB. Acquisition of data: AU, AT. Analysis and interpretation of data: AU, AT, MA-R. Drafting of manuscript: AU, AT, MA-R, ME-V, JF-D. Critical revision: AU, AT, ME-V, JF-D, BM, MA-R, AG, SV, SB.

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MINI REVIEW
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PONV management in patients with QTc prolongation on the EKG

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Postoperative nausea and vomiting (PONV) is a commonly encountered problem in surgical practice. It delays discharge from the post-anesthesia care unit, requires additional resources to treat, and may increase the morbidity in some patients. Many effective drugs are available to treat or prevent PONV, however many of these drugs have the potential to prolong the QTc on the electrocardiogram (EKG) and increase the risk of serious ventricular arrhythmias, in particular, torsade de pointes. The QTc prolongation may be a manifestation of a genetic mutation resulting in abnormal myocyte repolarization or it may be acquired and associated with the use of various medications, electrolyte disorders, and physiological conditions. Patients predisposed to QTc prolongation presenting for surgery constitute a challenging group, since many drugs commonly used for PONV management will put them at risk for perioperative serious arrhythmias. This is an important topic, and our mini-review is an attempt to highlight the problem, summarize the existing experience, and generate recommendations for safe management of PONV for patients, who are at increased risk of QTc prolongation and arrhythmias. Focused prospective studies will help to find definitive answers to the discussed problems and challenges and develop specific guidelines for clinical application.

Keywords: PONV, postoperative nausea and vomiting, QTc, anesthesia, perioperative period, torsade de pointes

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INTRODUCTION

Postoperative nausea and vomiting (PONV) is a common phenomenon complicating the postoperative course which may result in significant discomfort to the patient, delay the discharge, and increase the cost of treatment (Gan et al., 2020). Various drugs have been successfully used to prevent and treat PONV (**Table 1**). Many of these drugs share the potential to prolong the QTc on EKG, something that increases the risk for development of ventricular arrhythmias, specifically, Torsade de Pointes (TdP): a phenomenon first described in 1966 by Dessertenne (Charbit et al., 2005; Curigliano et al., 2009; Drew et al., 2010; Singh et al., 2018; Berul, 2020) Indeed, most of the episodes of QTc prolongation seen with the use of antiemetic drugs remain clinically silent, self-limited, and rarely require active intervention. Nevertheless, there is a group of patients with hereditary or acquired risk factors for development of long QTc and TdP, and these patients require special attention during the perioperative period. EKG monitoring and an action plan to promptly treat TdP and other arrhythmias, should they occur, is mandatory.

TABLE 1 | Drugs commonly used to prevent and treat PONV (Charbit et al., 2005; Curigliano et al., 2009; Marbury et al., 2009; Owczuk et al., 2009; Drew et al., 2010; Brygger and Herrstedt, 2014; Chu et al., 2014; Perkins et al., 2015; Tracz and Owczuk, 2015; Täubel et al., 2017; Hellström and Al-Saffar, 2018; Lai and Huang, 2018; Hansen, 2019; Staudt and Watkins, 2019; Berul, 2020; Gan et al., 2020).

Drug	Mechanism of action	Doses to treat/Prevent PONV	Timing	QTc prolongation	Adverse effects	Comments
Ondansetron	5-HT3-RA	4–8 mg	Near the end of surgery	Mild temporary QTc prolongation	Headache, fatigue, constipation or diarrhea, drowsiness, fever	Safe for PONV prophylaxis when used in recommended doses
Granisetron	5-HT3-RA	0.35–3 mg IV	Near the end of surgery	QTc prolongation less pronounced compared to ondansetron	Headache, constipation or diarrhea, weakness, fever, dizziness	Safe for PONV prophylaxis when used in recommended doses
Palonosetron	5-HT3-RA	0.075 mg IV	At induction	No significant effects on QTc	Headache, constipation, diarrhea, dizziness	No EKG studies were done during the time of cmax
Metoclopramide	D2RA, 5-HT3 RA, 5-HT4 RA	10–20 mg I.V.	Near the end of surgery	Moderate risk for QTc prolongation	Agitation, insomnia, restlessness, akathisia, extrapyramidal motor symptoms	Doses >20 mg are rarely administered to avoid the side effects. However, lower doses have a weaker antiemetic effect compared to other drugs
Amisulpride	D2RA, D3RA	5 mg	At induction	No significant effects on QTc	Mild increase in prolactin level	Doses used for PONV prophylaxis do not significantly prolong the QTc
Droperidol	D2RA	<= 1.25 mg I.V.	At induction or near the end of surgery	Mild QTc prolongation	Drowsiness, restlessness, hyperactivity, anxiety	Safe for PONV prophylaxis when used in recommended doses
Haloperidol	D2RA	0.5–1 mg I.V.	At induction or near the end of surgery	Mild QTc prolongation	Drowsiness, restlessness, hyperactivity, anxiety	Safe for PONV prophylaxis when used in recommended doses
Midazolam	Benzodiazepine receptors	1–2 mg I.V.	At induction of anesthesia	No significant effects on QTc	Sedation, amnesia	May not be appropriate for older patients
Ephedrine	Predominantly indirect adrenergic stimulant, α1 β1, and D1 direct agonist	0.5 mg/kg I.M.	Near the end of surgery	Mild QTc prolongation	Hypertension, tachycardia	May need precautions in patients with LQTS, who are sensitive to sympathetic stimulation
Dexamethasone	Glucocorticoid receptor agonist, possibly, neurokinine receptor antagonist, anti-inflammatory action	4–8 mg I.V.	At induction	No action of QTc interval	Potential risk of infections, hyperglycemia	Effective for prophylaxis, not treatment of PONV.
Promethazine	H1A, D2RA2, α1RA, NMDARA, MChRA	6.25–12.5 mg I.V.	May be used as a rescue drug in PACU.	Moderate risk of QTc prolongation	Sedation, dizziness, double vision, dry mouth	Lack of influence on transmural dispersion of repolarisation makes the risk of TdP very low. IV and IM, oral, and rectal administration is safe. SC injection may cause tissue damage
Aprepitant	NK1RA	40–80 mg P.O.	Preoperative	No action of QTc interval	Fatigue, diarrhea, dizziness, hiccups	More effective in reducing the incidence of postoperative vomiting rather than nausea
Fosaprepitant	NK1RA	150 mg I.V.	At induction. Infuse over 30 min	No action of QTc interval	Constipation, diarrhea, headache, neutropenia	
Propofol	Decrease in the rate of GABA dissociation from the GABAA receptor, possible 5-HT3-RA1 and D2RA2	20 mcg/kg/min I.V. 20 mg bolus I.V.	Throughout surgery End of surgery, PACU	No action of QTc interval No action of QTc interval	Sedation, respiratory depression	Short acting effect. May be used as a rescue medication
Scopolamine	MChRA	1.5 mg transdermal patch	Prior evening or 2 h before surgery	No action of QTc interval	Dry mouth, Visual disturbance, dizziness	Central and peripheral anticholonergic effects may limit the use in elderly
Gabapentin	Inhibition of voltage-gated calcium channels; GABAB receptor activation	600–800 mg P.O.	Preoperative	No action of QTc interval	Drowsiness, dizziness, drowsiness, blurred vision	The therapeutic effect against PONV is masked, when propofol is used during anesthesia

5-HT3-RA, 5-HT3 receptor antagonist; D2RA, Dopaminergic type 2 receptor antagonist; D3RA, Dopaminergic type 3 receptor antagonist; 5-HT4RA, 5-HT4 receptor agonist; H1A, histamine type 1 receptor antagonist; NK1RA, neurokinin 1 receptor antagonist; α1RA, alpha 1 receptor antagonist; NMDA RA, NMDA receptor antagonist; MChRA, M cholinergic receptor antagonist; QTc, QT corrected; PONV, postoperative nausea and vomiting; LQTS, long QT syndrome; TdP, Torsades de pointes; GABA, Gamma Aminobutyric Acid; GABAA, GABA A receptor; GABAB, GABA B receptor.

TABLE 2 | Types of LQTS (Curigliano et al., 2009; Drew et al., 2010; Staudt and Watkins, 2019).

		Genetic mutation	Prophylaxis and therapy[a]	Comments
LQTS 1	75%	Loss of function mutations in KCNQ1 (slowly activating component in the delayed rectifier K^+ current channel)	Beta blockers (very effective), $MgSO_4$ IV, treat hypokalemia, hypocalcemia, implantable devices, thoracic sympathectomy	May be induced by sympathetic activation
LQTS 2		Loss of function mutations in KCNH2 (responsible for the rapidly activating component of the delayed rectifier K + current channel)	Beta blockers, $MgSO_4$ IV (30 mg/kg), treat hypokalemia, hypocalcemia, implantable devices, thoracic sympathectomy	May be induced by sympathetic activation
LQTS 3		Gain of function mutations in SCN5A (responsible for encoding an inward sodium current channel)	Beta blockers (less effective), $MgSO_4$ IV, treat hypokalemia, hypocalcemia, avoid bradycardia	May be induced by sleep or rest with slowing of heart rate
Less common forms of LQTS	5%	ANKB, ANK2, mink, IsK, KCNE1, MiRP1, KCNE2, Kir2, KCNJ2, CACNA1C	Similar to LQTS 1 and 2	
Genetically silent congenital LQTS	20%	No mutations revealed	Similar to LQTS 1 and 2	

LQTS, long QT syndrome.
[a]*defibrillation, cardioversion or pacing should be used in cases when conservative measures are ineffective, and the patient develops cardiovascular instability.*

PROLONGED QTC AND ANESTHESIA

Abnormal cardiac repolarization is a common occurrence and is identified as prolonged QTc interval. QTc prolongation can be inherited, acquired or both. A significant number of patients present with QTc prolongation prior to anesthesia and surgery, even without any use of drugs with QTc prolonging properties, including antiemetic medications. It is well known that many patients who have a genetic predisposition to the long QT syndromes (LQTS) never develop a QTc prolongation, and additional contributing factors are required, including medications, to trigger ventricular arrhythmias (Curigliano et al., 2009; Drew et al., 2010). Nevertheless, these patients are considered a group of high risk, since their risk for sudden cardiac death is increased more than twofold and is even higher among younger patients (Brygger and Herrstedt, 2014). According to Staudt and Watkins. (2019), the estimated incidence of hereditary QTc prolongation among newborns is 1 in 2,500 (Staudt and Watkins, 2019).

Thus, despite the low chance of developing TdP or other life-threatening arrhythmias, patients with a preexisting QTc prolongation need proper anesthesia planning to minimize the risk of adverse events. It is important to emphasize that the risk of TdP rises significantly as various risk factors pile up. Preoperative risk assessment, treatment of correctible factors, and appropriate preventive measures will significantly decrease the incidence of life-threatening arrhythmias during the surgical procedure. Familiarity with potential complications, treatment options, and preventive measures are prerequisites for safe anesthesia management.

The QTc is most commonly measured using Bazett's formula ($QTc = QT/RR^{1/2}$), or Fridericia formula ($QTc = QT/RR^{1/3}$) (Drew et al., 2010). The latter one is preferentially used with higher heart rates. The upper limit of normal QTc is 470 msec in postpubertal males and 480 msec in postpubertal females (Berul, 2020). While measuring the QTc, one should keep in mind that there is a significant fluctuation of the interval during the day depending on the sympathetic activity and state of wakefulness (Brygger and Herrstedt, 2014). Values >480 msec on repeated testing or >460 msec in combination with a syncope will suggest LQTS (Staudt and Watkins, 2019). QTc >500 msec or an increase >60 msec is considered a significant risk factor for TdP and requires immediate intervention to avoid lethal arrhythmias (Brygger and Herrstedt, 2014). Additional findings in patients with LQTS include T-wave alterations, prolonged T_{peak}-T_{end} interval, and polymorphic ventricular arrhythmias (Staudt and Watkins, 2019). In patients with LQTS, the EKG picture may be variable and the clinical manifestations may vary from absent to life-threatening arrhythmias such as sudden cardiac arrest (Brygger and Herrstedt, 2014). It is noteworthy to mention that up to 40% of patients with genetic predisposition do not demonstrate QT prolongation on a resting electrocardiogram (ECG). (Staudt and Watkins, 2019).

Patients diagnosed with QTc prolongation belong to two major groups: congenital, and acquired QTc prolongation (Curigliano et al., 2009; Staudt and Watkins, 2019). This distinction is, however, arbitrary. Even though it is convenient to think of QTc prolongation as occurring because of either congenital or acquired reasons, the phenomenon may sometimes involve a gene–environment interaction (Curigliano et al., 2009). Genetic mutations in patients with LQTS entail defects in structural proteins of ion channels involved in myocardial action potential generation, with resultant channelopathy and prolonged myocardial repolarization. The delayed repolarization puts patients at risk for sudden onset of ventricular tachyarrhythmias, most notably TdP (Staudt and Watkins, 2019).

The first genetic mutations implicated in development of hereditary QTc prolongation were described in mid-1990s, and currently around 1000 mutations in twelve distinct LQTS susceptibility genes have been identified that contribute to congenital predisposition to LQTS (Drew et al., 2010; Staudt and Watkins, 2019). Patients, who become symptomatic early in their life, are at higher risk for life-threatening events, including sudden cardiac death. The most common types of congenital LQTS are shown in **Table 2**. The syndrome is seen in one out of

2,000–3,000 people, and it may follow both autosomal dominant and recessive pattern with variable penetrance (Brygger and Herrstedt, 2014; Staudt and Watkins, 2019).

The congenital forms of the disease mostly remain silent, but in 10–12% of patients they initially present as sudden cardiac death. When left untreated, the mortality rate will reach 21% within a year after the initial syncope (Staudt and Watkins, 2019). The morbidity and mortality rates, however, significantly improve with appropriate therapy including beta blockers, correction of electrolyte deficiencies, and implantation of cardioverter-defibrillators, when indicated.

Besides genetic factors, many physiological conditions, disease states, and medications may also predispose to long QTc (Drew et al., 2010). These factors include:

- ✓ hypomagnesemia,
- ✓ hypokalemia,
- ✓ hypocalcemia,
- ✓ bradycardia,
- ✓ sleep,
- ✓ drugs reducing the activity of hepatic enzymes and the rate of drug degradation
- ✓ female sex,
- ✓ older age,
- ✓ ischemic heart disease,
- ✓ ventricular hypertrophy.

Presence of these factors and concomitant use of drugs with QTc prolonging potential may predispose to acquired QTc prolongation and trigger arrhythmias in genetically susceptible patients. Preoperative correction of modifiable factors (items one to six in the list) is a mandatory requirement in planning anesthesia, which will significantly reduce the risk of severe arrhythmias during surgery.

When preparing for general anesthesia, it is important to review the patient's medication list to identify drugs with potential QTc prolonging property (Curigliano et al., 2009; Berul, 2020). A short list of such drugs include:

- ✓ class Ia and III antiarrhythmics,
- ✓ macrolide antibiotics,
- ✓ sulfamethoxazole-trimethoprim,
- ✓ milrinone,
- ✓ vasopressin,
- ✓ tricyclic antidepressants,
- ✓ phenothiazines,
- ✓ many chemotherapeutic agents,
- ✓ several cholinergic antagonists,
- ✓ diuretics.

In clinical setting, it is difficult to assess the incidence and the extent of QTc prolongation caused by a specific drug, since most patients receive multiple drugs with QTc prolonging potential. Their cumulative effect is more pronounced and, in general, stronger effects are seen with higher rates of infusion and at maximal plasma concentrations. In patients with long QTc, the lowest effective doses of the drugs should be used and administered as slow infusions rather than bolus injections. Such an approach will reduce the risk of severe arrhythmias.

Aside from various medications used to treat patients in the perioperative period, there are drugs routinely used as part of anesthesia, which may also prolong the QTc: succinylcholine, isoflurane, sevoflurane, and desflurane (Staudt and Watkins, 2019). These agents, whenever possible, should be avoided or used in reduced doses in high risk patients.

RELEVANCE OF QTC PROLONGATION TO PONV MANAGEMENT

PONV is a distressing symptom complex, commonly encountered in the postoperative period. The incidence of vomiting may reach 30%, while the incidence of nausea is around 50% among general surgical patients and up to 80% in high-risk groups (Gan et al., 2020).

Several scoring systems have been developed to assess the risk of PONV, and among them the Apfel score is the most popular one. It considers 4 parameters, which positively correlate with the risk of PONV:

1 Female gender,
2 Non-smoking status,
3 History of PONV,
4 Use of postoperative opioid analgesics.

Presence of each of the parameters adds roughly 20% to the risk of PONV.

Numerous drugs with antiemetic properties have been successfully used to prevent and treat PONV (**Table 1**). Unfortunately, many of these drugs and their pharmacological groups share the potential for QTc prolongation. Patients with acquired or congenital forms of QTc prolongation present a group of increased risk for development of serious arrhythmias during surgery. In these patients, safe anesthesia management with PONV prophylaxis and treatment requires understanding of the underlying mechanisms of QTc prolongation and careful risk - benefit assessment. For most of those patients, antiemetic therapy may still be safely used, despite the potential for a transient QTc prolongation, since the EKG changes are mostly self-limited and remain clinically silent. However, all patients will require preoperative correction of predisposing correctible factors, including electrolyte disorders, preferential use of antiemetic medications devoid of QTc prolonging properties, and preparedness to intervene should serious arrhythmias take place. Appropriate medications, defibrillation/cardioversion and pacing equipment should be readily available.

The following approach may help to safely provide anesthesia in patients with a long QTc.

Preoperative Period

Detailed family history of arrhythmias, episodes of syncope, and sudden death should be obtained. As part of preoperative

assessment, all the existing modifiable risk factors should be revealed and corrected prior to surgery, whenever possible. This will include:

- ✓ correction of electrolyte disorders,
- ✓ stabilization of cardiovascular function,
- ✓ a review of the medication list with exclusion, if possible, of drugs with QTc prolonging properties,
- ✓ establishment of an anesthesia plan which will include use of safe anesthetics, adequate cardiovascular monitoring, ensure availability of drugs and equipment to treat intraoperative arrhythmias,
- ✓ PONV prophylaxis with drugs and non-pharmacological methods with no arrhythmogenic potential.

Patients with long QTc, who are at low risk for PONV, probably, do not need any prophylactic antiemetic premedication to avoid unnecessary risk for rare but well-described side effects (Gan et al., 2020).

Patients with increased PONV risk may safely receive midazolam, gabapentin, amisulpride or a scopolamine patch, whenever appropriate. Palonosetron may also be used before surgery, since no cases of QTc prolongation have been reported with the drug (Gan et al., 2020). However, there are no studies done on QTc at the time of the drug's C_{max}. The NK_1 receptor antagonist aprepitant and its prodrug fosaprepitant (available for IV infusion) can safely be used in patients with long QTc (Marbury et al., 2009). Alternatively, preoperative acupuncture at the P6 point can be recommended as a non-pharmacological and safe method of PONV prevention. Again, it is important to correct any preexisting electrolyte deficiencies (hypomagnesemia, hypokalemia, hypocalcemia) before anesthesia. In case of time restrictions, corrective therapy should continue during surgery.

Intraoperative Period

Defibrillation/cardioversion and pacing equipment should be readily available during surgery. In patients with high risk for TdP and other serious arrhythmias, vapor anesthetics should be avoided or used with great caution. Patients with LQTS type 2 are more susceptible to vapor anesthetics, which may trigger serious arrhythmias in this group (Staudt and Watkins, 2019).

Total intravenous anesthesia with propofol and low dose opioids may be used safely. Alternatively, nitrous oxide and oxygen mixture with a low dose propofol (20–50 mcg/kg/min) infusion may be used in some patients, since nitrous oxide has minimal impact on PONV in low risk patients (Staudt and Watkins, 2019; Gan et al., 2020).

Dexamethasone can safely be used in all patients since it lacks any QTc prolonging properties. Dropridol, haloperidol, ondansetron and other drugs, with a potential for QTc prolongation, should be excluded in patients with QTc >480 mces. If QTc <480 msec and there is no history of arrhythmias or syncopes, droperidol (1.0–1.25 mg), or ondansetron (4–8 mg), may be used cautiously if diluted and injected slowly, since the risk of cardiac side effects of the drugs increases proportionally with the C_{max}.

There has been a long debate about droperidol related to the black box warning generated by the FDA regarding the risk for TdP (Kantor, 2002; Habib and Gan, 2003; Kao et al., 2003; Shafer, 2004; Charbit et al., 2005; Charbit et al., 2008; Halloran and Barash, 2010; Perkins et al., 2015; Tracz and Owczuk, 2015; Lai and Huang, 2018). It is well known that butyrophenones, including droperidol and haloperidol, increase the QTc, and in some patients such a transient prolongation may trigger TdP or other arrhythmias. However, the extent of QTc prolongation caused by droperidol, like many other drugs, is transient and dose-dependent. Charbit. et al. (2008) compared the maximal placebo time-matched and baseline-subtracted QTc prolongation for droperidol and ondansetron in 16 patients and found a small but statistically significant difference between the drugs: the QTc prolongation was 25 ± 8 msec for droperidol and 17 ± 10 m sec for ondansetron (Charbit et al., 2008). Interestingly, Charbit B. and coauthors published the results of another study in 2005, where 85 patients with PONV were included (Charbit et al., 2005). In that study, the authors described a mean maximal QTc interval prolongation of 17 ± 9 m sec after droperidol occurring at the second minute and 20 ± 13 msec after ondansetron at the third minute. The authors concluded that when used in treatment of postoperative nausea and vomiting, a situation where prolongation of the QTc interval seems to occur, the safety of 5-hydroxytryptamine type 3 antagonists may not be superior to that of low-dose droperidol (Charbit et al., 2005). The discrepancies in these papers as well as many others can be explained by differences in study designs, patient selection criteria and differences in drug doses studied. In the latter study, the authors used 0.75 mg of intravenous droperidol vs. 1 mg in the previous study. This indicates that lower doses of droperidol are safer for use, and their cardiac effects are minimal. According to the American Academy of Emergency Medicine position statement published in 2015, in the year 2000, over 25 million unit doses of droperidol were sold and only 10 adverse cardiac events were related to doses of 1.25 mg or less as cited in (Perkins et al., 2015). All 10 of patients had confounding factors that could have explained the cardiac event.

Currently, the IV Consensus Guidelines for the Management of Postoperative Nausea and Vomiting recommend intravenous droperidol (0.625 mg) for PONV management (Gan et al., 2020).

As a precaution, or as a first line therapy, $MgSO_4$ (30 mg/kg), and beta blockers, should be used if an arrhythmia is observed. $MgSO_4$ is indicated even in patients with normal serum magnesium levels. If not corrected in the preoperative period, hypokalemia and hypocalcemia should be treated promptly. Depending on specific clinical scenarios, additional antiarrhythmic drugs and cardioversion may be required to control the intraoperative episodes of arrhythmia.

Postoperative Period

If antiemetic treatment is required in the postoperative period, diluted slow injections of ondansetron, droperidol or haloperidol may be used in low risk patients. Small boluses of propofol may be used in high risk patients. Drugs with no QTc prolonging properties described in **Table 1** may be used as well.

It is important to minimize opioid administration to further reduce the risk of PONV, and non-opioid analgesics should be considered for pain control. EKG monitoring should be continued in high risk patients in the postoperative period.

CONCLUSION

Proper planning and preparation to treat adverse events, use of drugs with no or minimal QTc prolonging properties in the perioperative period can minimize the risks of long QTc and avoid life threatening arrhythmias. Additional studies and clinical trials will help to develop specific strategies to manage the perioperative nausea and vomiting is this group of high risk.

AUTHOR CONTRIBUTIONS

SS suggested the topic as a new one and deserving clarification, since there are not enough publications in the field. He reviewed the literature sources, wrote the manuscript and prepared the tables. He serves as the corresponding author for the submission. NS ran literature search and sorting. She participated in writing the manuscript and preparing the tables. WA ran literature search, participated in writing the manuscript and preparation of tables. SB supervised the process and distributed the roles. She participated in literature search, writing of the manuscript, and preparation of the tables. She made final editions as a cardiac anesthesiologist.

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Adverse Cardiovascular Effects of Phenylephrine Eye Drops Combined With Intravenous Atropine

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Background: Phenylephrine and atropine can cause serious adverse effects when applied in combination. We investigated the effect of phenylephrine eye drops combined with intravenous atropine on the cardiovascular system in patients under general anesthesia undergoing intraocular surgery.

Methods: The effects of the drugs were observed through clinical study. Thirteen patients undergoing intraocular surgery under general anesthesia were observed in this study; all were injected intravenously with atropine due to the oculocardiac reflex during surgery. To study the combination of drugs, an *in vivo* study was performed on rats. Seventy-two standard deviation rats that received phenylephrine eye drops and intravenous atropine treatment under general anesthesia were assessed, of which 18 treated with these drugs simultaneously were administered normal saline, neostigmine or esmolol. Blood pressure and heart rate were recorded and analyzed.

Findings: The age of the patients ranged from seven to 14 years old with an average age of 10.7 years old, and 11 patients were male. In patients, 5% phenylephrine eye drops combined with intravenous atropine led to a significant heart rate increase and the increase lasted 20 min. The significant increase in diastolic blood pressure and systolic blood pressure lasted for 15 and 25 min, respectively. From five to 25 min after intravenous atropine treatment, the systolic blood pressure and diastolic blood pressure were both more than 20% higher than that at baseline. In rats, the changes in blood pressure and heart rate were independent of the phenylephrine and atropine administration sequence but were related to the administration time interval. The neostigmine group showed a significant decrease in blood pressure after the increase from the administration of phenylephrine and atropine.

Interpretation: Phenylephrine eye drops combined with intravenous atropine have obvious cardiovascular effects that can be reversed by neostigmine. This drug combination should be used carefully for ophthalmic surgery, especially in patients with cardio-cerebrovascular diseases.

Keywords: phenylephrine, atropine, Combination, neostigmine, adverse cardiovascular effects, hypertension emergency

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INTRODUCTION

Agonism of alpha 1-adrenoceptors in the iris dilator leads to mydriasis. Phenylephrine is a selective alpha 1-adrenergic agonist. It can produce prompt and transient mydriasis without increasing the intraocular pressure or affecting the accommodative ability of the eye (cycloplegia) (Esteve-Taboada et al., 2016). Phenylephrine (1%–10%) is often used as a mydriatic agent to facilitate ophthalmic examination and surgery in clinical practice (Stavert et al., 2015). Phenylephrine eye drops can cause systemic side effects, including blood pressure elevation and heart rate alterations, via absorption through the conjunctiva and nasal mucosa (Fraunfelder et al., 2002; Alpay et al., 2010). However, a systematic review and meta-analysis revealed that the systemic side effects of phenylephrine may have been overstated over the years (Stavert et al., 2015). The review showed that 2.5% phenylephrine does not cause clinically significant changes in blood pressure or heart rate in either neonates or adults, and 10% phenylephrine may lead to hemodynamic instability in infants but has no adverse effects in adults due to transient and recoverable changes in blood pressure and heart rate (Stavert et al., 2015). Moreover, these changes in adults are innocuous, similar to circadian or postural blood pressure changes (Stavert et al., 2015).

Ophthalmic surgery operations that stimulate eyeball or ocular tissue can induce the oculocardiac reflex (OCR) directly, and OCR incidence is high during surgery (Aletaha et al., 2016). Serious side effects due to OCR, such as bradycardia, arrhythmia and even cardiac arrest, can occur during eye surgery, especially during corrective strabismus surgery (Deb et al., 2001). Opioids are often used to provide analgesia during the induction and maintenance of general anesthesia (Cravero et al., 2019). The heart rate can be slowed due to the activation of opioid-specific receptors (Chen and Ashburn, 2015). Atropine is an antimuscarinic agent that can compete with acetylcholine for the acetylcholine receptor, thus antagonizing the muscarine-like actions of acetylcholine (Blumenberg, 1987). Atropine is administered as premedication during general anesthesia. Intravenous atropine treatment is often used clinically to inhibit glandular secretion and relieve severe bronchospasm under general anesthesia. More notably, atropine is a very useful drug for preventing or reducing the incidence of sinus bradycardia or bradycardia caused by OCR or opioid applications in eye surgery (Klockgether-Radke et al., 1993; Gilani et al., 2005; Sajedi et al., 2013; Ho et al., 2018). However, atropine cannot totally prevent bradycardia but rather may cause bigeminy and increase ectopic beats, which are more persistent than the OCR (Espahbodi et al., 2015). Generally, atropine is also used as a cycloplegic to temporarily paralyze the accommodation reflex and as a mydriatic to dilate the pupils.

Phenylephrine eye drops and intravenous atropine treatment are often used together in ophthalmic surgeries while patients are under general anesthesia in clinical practice. Although adverse cardiovascular reactions following phenylephrine instillation or intravenous atropine treatment have been reported, the adverse effects and interaction mechanisms of the combination of the two drugs are unclear. Notably, we first noticed a phenomenon in clinical practice where concurrent application of phenylephrine eye drops and intravenous atropine during eye surgery can cause clinically significant increases in heart rate and blood pressure, which could even be life-threatening without intervention. This phenomenon prompted us to think and consult the relevant literature. Keys and Violante reported that subcutaneously injected atropine followed by subcutaneously injected phenylephrine caused a higher blood pressure and heart rate than phenylephrine alone due to atropine enhancing the pressor effect of phenylephrine (Keys and Violante, 1942). Levine and Leenen found that after intravenous atropine treatment the blood pressure responses to intravenous phenylephrine treatment were markedly potentiated in normotensive volunteers (Levine and Leenen, 1992). Fraunfelder and Scafidi found that concomitant use of phenylephrine eye drops and atropine eye drops can cause blood pressure elevation and induce tachycardia in some patients (Fraunfelder and Scafidi, 1978). Tropicamide is a synthetic muscarinic antagonist with actions similar to those of atropine. Co-administration of phenylephrine and tropicamide eye drops may lead to severe adverse effects (Pescina et al., 2017). The compound tropicamide eye drop, a compound preparation containing tropicamide and deoxyepinephrine hydrochloride, is commonly used in China as a routine mydriatic agent for fundus examination and refractive examination (Li et al., 2016). Through a literature review, we also found that some cases of systemic adverse reactions associated with the use of compound tropicamide eye drops have been reported. Sbaraglia et al. reported the occurrence of complications in pediatric patients undergoing ophthalmic surgery after the application of a mydriatic agent of the compound tropicamide and attributed the causes to the alpha 1-adrenergic action of phenylephrine, which we think may dismiss the adverse effects of the combination of the two drugs (Sbaraglia et al., 2014). The mechanism and the solutions to the effects of concurrent use of phenylephrine eye drops and intravenous atropine treatment have not been fully discussed; therefore, ophthalmologists do not pay enough attention to these effects, and similar problems have been occurring in clinical work for a long time. Therefore, through our observation of 13 patients and corresponding animal experiments, we hope to attract more attention from ophthalmologists and anaesthetists to prevent similar problems from occurring in the future. The aim of the study was to assess the adverse cardiovascular effects of the concurrent application of phenylephrine eye drops and intravenous atropine during ophthalmic surgery. Another purpose of this paper was to study the mechanism of adverse cardiovascular effects and the corresponding prevention and treatment interventions.

MATERIALS AND METHODS

Patients and Clinical Observation

This retrospective analysis included 13 patients who received 5% phenylephrine eye drops combined with intravenous atropine administration during intraocular surgery while under general anesthesia at the Zhongshan Ophthalmic Center from 2016 to 2019. The studies were reviewed and approved by Medical Ethics

Committee of Zhongshan Ophthalmic Center, Sun Yat-sen University. The age of the patients ranged from seven to 14 years old with an average age of 10.7 years old, and 11 patients were male. The induction of general anesthesia was performed using midazolam (0.03 mg/kg), fentanyl (1 μg/kg), atropine (5 μg/kg), propofol (2–3 mg/kg), and vecuronium (0.6 mg/kg). A tracheal tube was inserted approximately 5 min after induction, and propofol (150–200 μg/kg/minute) and remifentanil (0.2 μg/kg/minute) were used to maintain anesthesia. Eye surgery began approximately 10 min after the instillation of 10–20 mg phenylephrine eye drops. The traction of extraocular muscles or compression to the eyeball can cause a temporary decrease in heart rate, known as OCR. If the heart rate (beats/minute, bpm) decreases by more than 10% from the base heart rate, surgical procedures should be stopped, and the traction of the extraocular muscles or compression of the eyeball should be relaxed (Espahbodi et al., 2015). If the OCR is still not recovered within 20 s, 5 μg/kg atropine should be injected intravenously in routine clinical practice (Espahbodi et al., 2015).

In our study, the heart rate, systolic blood pressure (SBP) and diastolic blood pressure (DBP) of all patients were recorded at different points, namely, before anesthesia, immediately after anesthesia induction, and 5, 10, 15, 20, 25, and 30 min after intravenous atropine treatment.

Reagents

A 0.9% sodium chloride injection (lot number: C180705D) was purchased from Hubei Kelun Pharmaceutical Co., Ltd (Hubei, China). Pentobarbital sodium (lot number: 4,579/50) was purchased from Tocris Cookson Ltd (Bristol, United Kingdom). The 5% phenylephrine eye drops (lot number: 180605) were purchased from Zhongshan Ophthalmic Center, Sun Yat-sen University (Guangdong, China). Atropine sulfate injection (lot number: 1802024) was purchased from Henan Runhong Pharmaceutical Co., Ltd. (Henan, China). Tetracaine hydrochloride eye drops (lot number: 180524) were purchased from Zhongshan Ophthalmic Center, Sun Yat-sen University (Guangdong, China). Esselor hydrochloride injection (lot number: 7B0062005) was purchased from Qilu Pharmaceutical Co. Ltd (Shandong, China). Neostigmine methylsulfate injection (lot number: 1710605) was purchased from Shanghai Xinyi Jinzhu Pharmaceutical Co., Ltd (Shanghai, China).

Animals and Study Design

Seventy-two wild-type Sprague-Dawley (SD) rats with body weights ranging from 180 to 250 g were purchased from Hunan SJA Laboratory Animal Co., Ltd (Hunan, China). The use of experimental animals in this study complied with the animal ethics principles, and the protocol was approved by the laboratory animal ethics committee (No. 00201878). Based on previous research and animal welfare, we tried to reduce the number of animals we used as long as we could achieve our experimental objectives. To obtain accurate analysis and minimize the number of experimental animals, a proper number of experimental animals was used. The experimental rats were randomly divided into 12 groups using a random number table, with six rats in each group. Specifically, group A received 5% phenylephrine eye drops only; group B received intravenous atropine administration only; group C received phenylephrine and atropine simultaneously; groups D, E, and F received 5% phenylephrine eye drops first and then received intravenous atropine treatment five, ten, and 15 min later, respectively,; groups G, H, and I received intravenous atropine administration first followed by 5% phenylephrine eye drops 5, 10, and 15 min later, respectively,; group J received phenylephrine and atropine simultaneously followed by intravenous saline treatment 1 min later; and groups K and L received phenylephrine and atropine simultaneously followed by 0.15 mg/kg neostigmine and 1.0 mg/kg esmolol treatment 1 min later, respectively.

The rats were anesthetized with an injection of 1% pentobarbital sodium (50 mg/kg). Before the procedure, all catheters were heparinized with 1000 IU/ml heparin to empty the air in the device. The left common carotid artery was isolated while the rats were in the supine position. Arterial cannulas were placed in the common carotid artery of rats and connected to a physiological monitor. Parameters were measured in this study using the BL-420 F biological signal acquisition and analysis system (Chengdu Taimeng Science and Technology Co. Ltd.). After surface anesthesia of the right eye with tetracaine hydrochloride eye drops, a T-shaped incision of conjunctiva approximately 2 mm away from the corneal rim was created by creating an incision approximately 0.3 cm along the corneal rim and then cutting approximately 0.3 cm away from the cornea. Finally, one 5% phenylephrine eye drop was added to the incision. In addition, 0.06 mg/kg atropine, 0.15 mg/kg neostigmine and 1.0 mg/kg esmolol were administered via tail vein injection. For simultaneous administration of phenylephrine and atropine, the right eye was anesthetized and operated first, followed by 0.06 mg/kg intravenous atropine treatment and the administration of one 5% phenylephrine eye drop.

The operations were performed according to the corresponding time intervals based on different groups. The blood pressure and heart rate were recorded 5 min before any treatment until 40 min after treatment.

Statistical Analysis

Data are all shown as the mean ± standard deviation (SD). The statistical methods used in this study were an analysis of variance (ANOVA) for the comparison of means among groups and Student's t test for the comparison of means between two groups. SPSS 26.0 (SPSS, lnc., Chicago, IL, United States) was used to analyze the data, and all experiments were blinded. A p value was considered statistically significant when it was less than 0.05.

RESULTS

Changes in the Heart Rate and Blood Pressure of Patients

The heart rate was significantly higher 10 min after intravenous atropine treatment than that at baseline (ANOVA, 95%

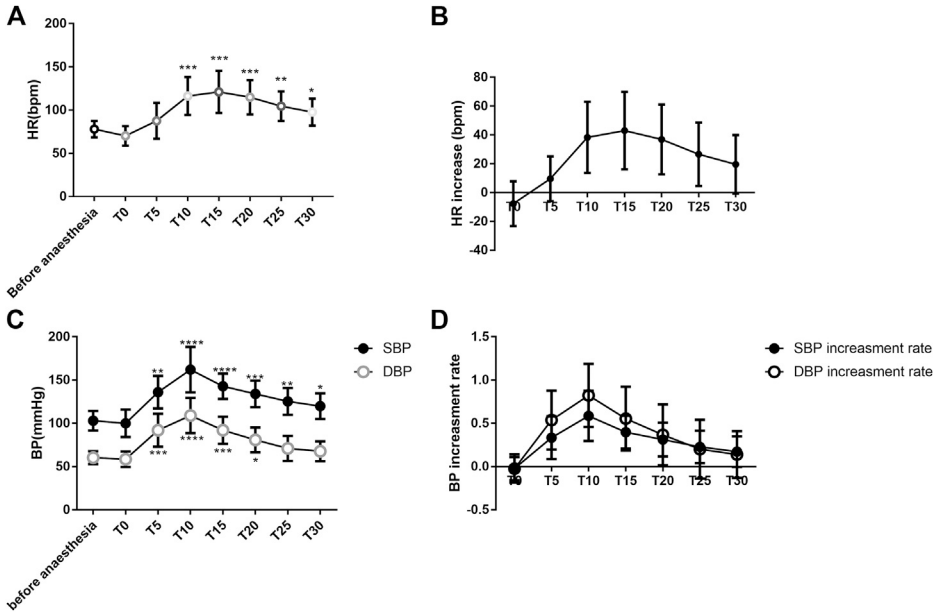

FIGURE 1 | Changes in the heart rate and blood pressure of patients **(A)**. Changes in HR associated with anesthesia and intravenous atropine treatment; **(B)**. Increase in HR associated with anesthesia and intravenous atropine treatment; **(C)**. Changes in blood pressure associated with anesthesia and intravenous atropine treatment; **(D)**. Increase in blood pressure associated with anesthesia and intravenous atropine treatment. Error bars represent the mean ± SD; the comparison was performed using one-way ANOVA. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate. T0, immediately after anesthesia induction; T5, T10, T15, T20, T25, and T30, representing five, ten, 15, 20, 25, and 30 min after intravenous atropine treatment, respectively. N = 13. *$p < 0.05$, **$p < 0.01$, and ****$p < 0.0001$ compared with the before anesthesia group. N = 13.

confidence interval (CI) = −60.39 to −16.22 bpm, $p = 0.0008$). The peak heart rate occurred 15 min after intravenous atropine treatment (ANOVA, 95% CI = −67.11 to −19.04 bpm, $p = 0.0006$) and slowly decreased until 30 min after treatment, but the heart rate was still significantly higher than that at baseline (ANOVA, 95% CI = −37.91 to −1.475 bpm, $p = 0.0311$) (**Figure 1A**). Topical ocular application of 5% phenylephrine and intravenous treatment with atropine led to a 43.08 ± 26.88 beat/minute increase in heart rate 15 min after intravenous atropine treatment compared with the heart rate before anesthesia (**Figure 1B**).

At 5 min after intravenous atropine treatment, SBP began to increase and was significantly different from the level before anesthesia (ANOVA, 95% CI = −53.28 to −12.72 mmHg, $p = 0.0014$). The highest SBP occurred 10 min after intravenous atropine treatment (ANOVA, 95% CI = −84.64 to −33.36 mmHg, $p < 0.0001$) and decreased slowly through 30 min after the treatment. SBP was still significantly higher than that before anesthesia (ANOVA, 95% CI = −32.56 to −0.8293 mmHg, $p = 0.00368$). SBP increased from 103.15 ± 11.38 mmHg before anesthesia to 162.15 ± 26.42 mmHg 10 min after intravenous atropine treatment. (**Figure 1C**). At 5 min after intravenous atropine treatment, DBP was significantly different than the preoperative level (ANOVA, 95% CI = −48.64 to −14.75 mmHg, $p = 0.0004$). The highest DBP appeared 10 min after administration of the medication (ANOVA, 95% CI = −66.8 to −30.43 mmHg, $p < 0.0001$), decreased gradually to the baseline level at 25 min with no difference (ANOVA, 95% CI = −27.22 to 6.144 mmHg, $p > 0.3724$) and remained stable at 30 min after administration of medication. Intravenous atropine treatment caused DBP to increase to the highest level, reaching 109.08 ± 20.41 mmHg from 60.46 ± 7.61 mmHg before anesthesia. (**Figure 1C**). The significant increase in blood pressure occurred 5 min after intravenous atropine treatment and was sustained for 15 or 25 min (**Figure 1C**). From five to 30 min after intravenous atropine treatment, the changes in SBP and DBP were all greater than 16.69 ± 17.74 and 7.31 ± 14.60 mmHg, respectively, (**Figure 1C**). From five to 25 min after intravenous atropine treatment, SBP and DBP were both more than 20% higher than the baseline blood pressure levels (**Figure 1D**).

Changes in the Blood Pressure and Heart Rate of Rats

Under anesthesia, no significant differences were found in SBP, DBP, mean arterial pressure (MAP), or heart rate among the groups (ANOVA, F = 0.6178, $p = 0.7582$; ANOVA, F = 1.6, $p = 0.1519$; ANOVA, F = 1.241, $p = 0.2984$; ANOVA, Bartlett's statistic (corrected) = 8.33, $p = 0.4019$) (**Figure 2**). The blood pressure and heart rate of the rats increased to a certain extent after the administration of phenylephrine or atropine alone (**Figure 2**). After applying phenylephrine 0, five, ten, and 15 min later, the combined use of intravenous atropine resulted in elevated blood pressure and heart rate compared with the values prior to the administration of medication (**Figure 2**). After intravenous atropine treatment five, ten, and

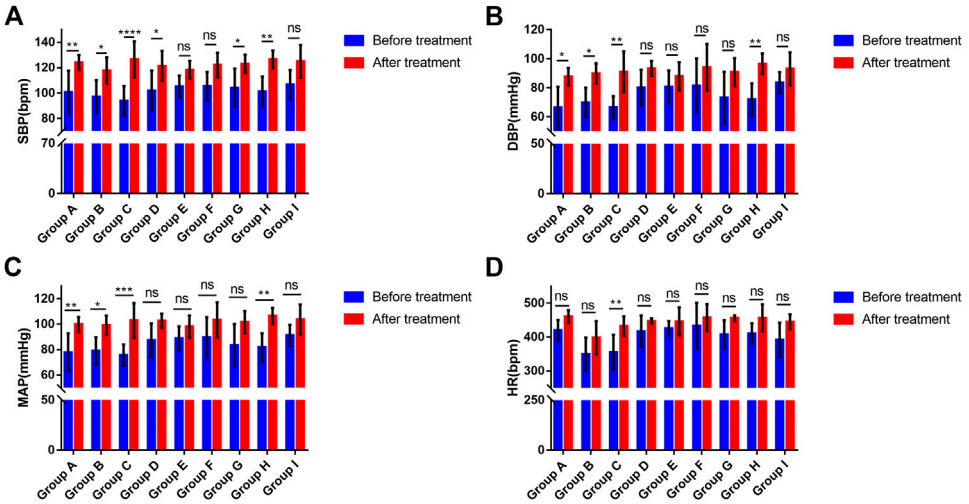

FIGURE 2 | Changes in the blood pressure and heart rate of rats **(A)**. Changes in SBP associated with phenylephrine eye drops and (or) intravenous atropine treatment; **(B)**. Changes in DBP associated with phenylephrine eye drops and (or) intravenous atropine treatment; **(C)**. Changes in MAP associated with phenylephrine eye drops and (or) intravenous atropine treatment; **(D)**. Changes in HR associated with phenylephrine eye drops and (or) intravenous atropine treatment. Error bars represent the mean ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate. Before treatment, blood pressure and heart rate before the administration of phenylephrine and (or) atropine. After treatment, the highest blood pressure and heart rate during the observation period. Group A received phenylephrine eye drops. Group B received intravenous atropine treatment. Group C received phenylephrine and atropine simultaneously. Groups D, E, and F received phenylephrine eye drops first and then intravenous atropine treatment five, ten, and 15 min later, respectively. Groups G, H, and I received intravenous atropine treatment first followed by phenylephrine eye drops five, ten, and 15 min later, respectively. N = six.

TABLE 1 | Variations in BP and HP after phenylephrine eye drop and intravenous atropine administration in rats.

	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	HR (bpm)
Group A	23.51 ± 16.20	21.17 ± 14.25	21.96 ± 14.73	40.33 ± 44.58
Group B	20.60 ± 10.66	19.98 ± 6.81	20.19 ± 7.43	48.67 ± 59.76
Group C	32.62 ± 13.58	24.44 ± 18.18	27.28 ± 16.01	77.17 ± 57.72
Group D	19.58 ± 11.22	13.21 ± 10.53	15.22 ± 10.67	30.33 ± 45.17
Group E	13.14 ± 8.73	7.34 ± 3.38	9.27 ± 4.63	20.50 ± 40.32
Group F	16.68 ± 8.45	12.61 ± 9.14	13.97 ± 8.45	24.83 ± 33.98
Group G	19.01 ± 10.29	17.67 ± 12.68	18.11 ± 11.19	48.00 ± 40.35
Group H	25.20 ± 15.32	24.59 ± 16.84	24.79 ± 16.25	46.00 ± 30.38
Group I	18.25 ± 7.61	9.52 ± 7.34	12.43 ± 7.30	53.33 ± 59.60

Error bars represent the mean ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HP, heart rate. Group A received phenylephrine eye drops. Group B received intravenous atropine treatment. Group C received phenylephrine and atropine simultaneously. Groups D, E, and F received phenylephrine eye drops first and then intravenous atropine treatment five, ten, and 15 min later, respectively. Groups G, H, and I received intravenous atropine treatment first followed by phenylephrine eye drops five, ten, and 15 min later, respectively. N = six.

15 min later, the phenylephrine eye drops also caused elevated blood pressure and heart rate compared with the values prior to the administration of medication (**Figure 2**). However, the changes in blood pressure and heart rate reached their peak when phenylephrine and atropine were administered simultaneously (**Table 1**). The increases in SBP, DBP, and MAP were 32.62 ± 13.58, 24.44 ± 18.18, and 27.28 ± 16.01 mmHg, respectively, and the increase in heart rate was 77.17 ± 57.72 bpm following the administration of both drugs simultaneously (**Table 1**). With the increase in time interval, the changes in blood pressure and heart rate started to decrease gradually (**Table 1**, **Figure2**). The changes in blood pressure and heart rate were independent of the sequence of administration of phenylephrine and atropine but related to the time interval between them. When the time interval between the administration of these two drugs was ten or 15 min, the increases in blood pressure and heart rate were the smallest (**Table 1**, **Figure 2**). The results of this experiment suggest that the combined application of phenylephrine and atropine has a synergistic effect of increasing blood pressure and heart rate.

Effects on Blood Pressure and Heart Rate in Rats After Neostigmine Treatment

Under anesthesia, no significant differences in SBP, DBP, MAP, or heart rate were observed among the groups (ANOVA, F = 0.2181, $p = 0.8065$; ANOVA, F = 0.57, $p = 0.5773$; ANOVA, F = 0.4008, $p = 0.6767$; ANOVA, F = 3.356, $p = 0.0624$) (**Figure 3**). Compared with the normal saline group, SBP, DBP and MAP significantly decreased in the neostigmine group (ANOVA, 95% CI = 12.54–51.72 mmHg, $p = 0.0002$; ANOVA, 95% CI = 13.69–55.09 mmHg, $p = 0.0002$; ANOVA, 95% CI = 14.58–52.7 mmHg, $p < 0.0001$). The mean heart rate decreased in the neostigmine group (**Figure 3**). However, the variations in heart rate of the neostigmine group were not significantly different compared with the normal saline group (t test, 95% CI = −131.9 to 33.88 bpm, $p = 0.2174$) (**Table 2**). Compared with the normal saline group, SBP, DBP, MAP, and HR were not significantly different in the esmolol group (ANOVA, 95% CI = −3.817–35.36 mmHg, $p = 0.2119$; ANOVA, 95% CI = −9.34–32.06 mmHg, $p = 0.7619$; ANOVA, 95% CI = −6.226–31.89 mmHg, $p = 0.4625$; ANOVA, 95% CI = −36.28 to 100.9 bpm, $p = 0.9038$) (**Figure 3**). Compared with the normal

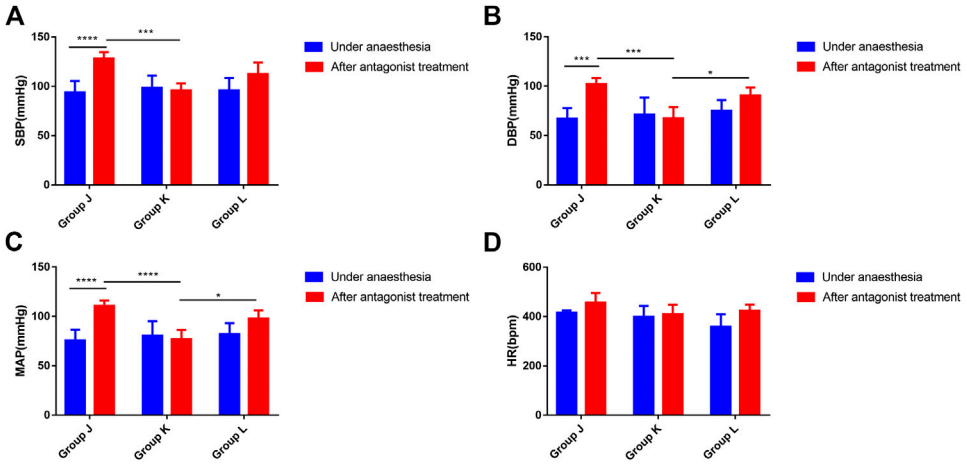

FIGURE 3 | Effects on blood pressure and heart rate in rats after neostigmine or esmolol treatment **(A)**. Changes in SBP associated with the neostigmine or esmolol treatment; **(B)**. Changes in DBP associated with the neostigmine or esmolol treatment; **(C)**. Changes in MAP associated with the neostigmine or esmolol treatment; **(D)**. Changes in HR associated with the neostigmine or esmolol treatment. Error bars represent the mean ± SD, and the comparison was performed using two-way ANOVA. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate. Under anesthesia, the blood pressure and heart rate under anesthesia. After antagonists, the highest blood pressure and heart rate within 40 min of using antagonists. Group J received phenylephrine and atropine simultaneously followed by intravenous saline treatment 1 min later. Groups K and L received phenylephrine and atropine simultaneously followed by 0.15 mg/kg neostigmine and 1.0 mg/kg esmolol treatment 1 min later. *$p < 0.05$, **$p < 0.01$. N = six.

TABLE 2 | Variations in BP and HP after neostigmine treatment in rats.

	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	HR (bpm)
Group J	34.44 ± 16.03	35.02 ± 15.98	34.83 ± 14.86	40.33 ± 86.46
Group K	−7.65 ± 6.97***	−11.53 ± 13.23***	−10.24 ± 11.04***	−8.67 ± 28.74
Group L	10.11 ± 16.55*	6.73 ± 16.21*	7.82 ± 16.12*	77.67 ± 62.36

Error bars represent the mean ± SD, and the comparison was performed using a t *test. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HP, heart rate. Group J received phenylephrine and atropine simultaneously followed by intravenous saline treatment 1 min later. Groups K and L received phenylephrine and atropine simultaneously followed by 0.15 mg/kg neostigmine and 1.0 mg/kg esmolol treatment 1 min later, respectively. ***p < 0.001, compared with the normal saline group. N = six.*

saline group, the heart rate increased in the esmolol group, and the variations in heart rate were not significantly different (*t* test, 95% CI = −59.63 to 134.3 bpm, $p = 0.4110$) (**Table 2**). The heart rate decreased to 409.50 ± 38.96 bpm after neostigmine treatment, which was lower than in the normal saline group (456.5 ± 39.1) and the esmolol group (424.17 ± 24.80) (**Figure 3**).

DISCUSSION

Based on clinical observations and animal experiments, we found hazardous effects of the combination of topical phenylephrine and intravenous atropine treatment on blood pressure and heart rate. In pediatric patients, five to 25 min after intravenous atropine treatment SBP and DBP were more than 20% higher than the baseline blood pressure level. Acute elevated blood pressure (SBP, DBP, or MAP over 20% of the baseline level) during the intraoperative period can be considered a hypertension emergency (Goldberg and Larijani, 1998). A hypertensive emergency is a serious life-threatening clinical syndrome with progressive acute impairment of the heart, brain, kidney, and other important target organs (Varon and Marik, 2008; Muiesan et al., 2015). In our cases, clinically high blood pressure and tachycardia commonly occurred in normotensive patients under anesthesia. Patients with a previous history of hypotension were prone to perioperative blood pressure fluctuations when using phenylephrine (Robertson, 1979). Hypertensive crizes were more likely to occur in patients with hypertension, especially when SBP was >180 mmHg or DBP was >110 mmHg (Aronson et al., 2002; Varon and Marik, 2008). More attention should be paid to such patients when phenylephrine instillation and intravenous atropine are used together. Animal experiments showed that the increase in both blood pressure and heart rate can be reduced by implementing longer time intervals between the administration of the two drugs. The maximum plasma levels from phenylephrine eyedrop administration are achieved within ten to 20 min after topical instillation (Kumar et al., 1985). Consistent with our findings from animal experiments, we suggest that using intravenous atropine after prolonged time periods (10 or 15 min after phenylephrine eye drops) could reduce the increase in blood pressure and heart rate. Tachycardia is a heart rate that exceeds the normal resting rate; it is a common event in patients under anesthesia, and it is often associated with hypertension. Intraoperative tachycardia was associated with poorer outcomes, such as increased mortality and likelihood of intensive care unit admission and prolonged hospital stay (Reich et al., 2002; Hartmann et al., 2003). When children are ten years old, the normal heart rate is 90 bpm and can vary from 70 to 110 bpm.

Tachycardia in ten-year-old children is considered when the heart rate is over 90 bpm while the patient is under anesthesia. Among our clinical cases, the heart rate began to increase significantly 10 min after administration of medication and the increase lasted for 20 min. The mean heart rate was over 90 bpm from ten to 30 min after intravenous atropine treatment. The tachycardia lasted for 20 min. The animal experiments showed that the heart rate increased by 20.50 ± 40.32 to 77.17 ± 57.72 bpm after the concurrent use of atropine and phenylephrine at different interval times.

Hypertensive emergencies usually require the intravenous administration of antihypertensive drugs (Kurnutala et al., 2014). Esmolol is a super short-acting selective beta antagonist. It can be used when the blood pressure and heart rate increase during surgery. Atropine could act competitively on M receptor with acetylcholine and thus heart rate could accelerate by inhibition of the excitatory tone of the vagal nerve. Neostigmine reversibly inhibits the activity of cholinesterase, thus increasing the concentration of acetylcholine at the receptor site in the body, strengthening and prolonging the effect of acetylcholine. Animal experiments have shown that neostigmine could significantly reduce blood pressure after they were increased due to the combined use of intravenous atropine treatment and phenylephrine instillation. Following the use of neostigmine, SBP and DBP decreased 7.65 ± 6.97 and 11.53 ± 13.23 mmHg, respectively. The heart rate decreased to 409.50 ± 38.96 bpm after neostigmine treatment, which was lower than that of the normal saline group after antagonist treatment. Meanwhile, SBP, DBP, MAP and HR did not decrease significantly in the esmolol group compared with the normal saline group. In our clinical cases, reducing the blood pressure and controling the heart rate by increasing the depth of anesthesia, increasing analgesia, and using beta blockers were difficult. We found that neostigmine could transiently improve the hemodynamic state while strengthening and prolonging the effect of acetylcholine. Clinical observations and animal experiments have indicated that neostigmine could distinctly reduce blood pressure and heart rate. Atropine may have played a leading role in this effect. In conclusion, neostigmine may be a safe and effective choice for reducing the blood pressure and heart rate in pediatric patients following the combined use of intravenous atropine treatment and phenylephrine instillation.

However, our clinical study had several limitations. A small sample size and the single-centred retrospective study are the major limitations of our study. In addition, this study only focused on pediatric patients and had a limited number of patients (more boys), resulting in considerably biased clinical features. A further retrospective study is needed in multiple centers with a large sample to observe the adverse cardiovascular effects of phenylephrine eye drops combined with intravenous atropine in patients.

CONCLUSION

We suggest the following recommendations for the clinical use of atropine and phenylephrine in pediatric patients:

1. The combined use of phenylephrine and atropine eye drops should be avoided.
2. Intravenous atropine can be used only for blunting OCR after a certain time interval, usually more than 10 min, after using phenylephrine eye drops.
3. The patient can be rescued with neostigmine if the concurrent use of intravenous atropine and phenylephrine eye drops results in clinically significant high blood pressure and heart rate.

To avoid serious adverse reactions, the following points should be noted for the clinical use of compound tropicamide eye drops:

1. Patients must be asked about their medical history and drug allergy history. Patients with cardiovascular disease should be treated with caution.
2. The nasolacrimal passage should be compressed for 60 s immediately following topical administration of compound tropicamide eye drops to minimize drainage.
3. Patients should be closely observed for systemic adverse reactions.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethics Committee of Zhongshan Ophthalmic Center, Sun Yat-sen University. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements. The animal study was reviewed and approved by The laboratory animal ethics committee of Drug Evaluation Center, Guangzhou Boji Pharmaceutical Biotechnology Co., Ltd (No. 00201878).

AUTHOR CONTRIBUTIONS

WG conceived and designed the study and provided the clinical data. YG, YW, and HZ performed the animal experiments. YG, QL, SZ and JT performed the analyses. YW, JP, and XY conducted the analyses. QL and YD wrote the paper. WG and WC reviewed and edited the manuscript. All authors read and approved the manuscript.

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CASE REPORT
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Case Report: Post-operative Angioedema After a Laryngeal Mask Airway Application

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Angioedema with macroglossia is a rare complication of anesthesia. We present a clinical case of post-operative development of angioedema presenting as macroglossia in a patient receiving chronic therapy with lisinopril, who developed symptoms in the early post-operative period following surgery in a lateral position, when a laryngeal mask airway was used. Possible mechanisms of angioedema and macroglossia development in our patient are discussed along with potential underlying predisposing mechanisms and available therapeutic approaches.

Keywords: angioedema, quincke's edema, anesthesia complications, lisinopril, laryngeal mask airway

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INTRODUCTION

Angioedema (AE) or Quincke's edema is an acute-onset transient edema involving the skin, subcutaneous tissues, and mucous membranes of the face, oral cavity, airway structures or the gastrointestinal tract, the upper and lower extremities (1–5). The underlying mechanisms include histamine and inflammatory cytokine release, overproduction or decreased degradation of bradykinin, or a hereditary C1 esterase inhibitor deficiency: a rare disease with autosomal dominant inheritance (1, 4).

AE related to histamine release presents as an allergic reaction of an immediate type triggered by IgE-mediated release of histamine and other mediators by mast cells and basophils.

Cases of AE with increased levels of bradykinin are generally associated with use of specific medications: angiotensin converting enzyme (ACE) inhibitors, angiotensin 2 receptor antagonists, non-steroidal anti-inflammatory medications, and other drugs, including propofol, which increase bradykinin levels in tissues (1, 3, 4, 6–9). According to Ishoo E. and co-authors, ACE inhibitors are the most common cause of drug-induced AE representing 25–39% of cases (4).

Typically, propofol-induced bradykinin release is restricted to the site of injection and manifests as a transient burning sensation during drug administration. Other contributing factors include anxiety, pain, significant physical and surgical stress, infections, and temperature changes (3).

The hereditary AE due to C1 esterase inhibitor deficiency is seen in 1 in 50,000 people in the general population (10). Acquired forms of C1 esterase inhibitor deficiency have been reported and are usually associated with malignant B-cell lymphoma or several other conditions.

Literature reports describe development of AE during surgery or in the immediate post-operative period. Perioperative AE, especially if the soft tissues of face, neck, oropharynx and airway are involved, is a rare but serious complication which may require continuous monitoring and sometimes, prompt intervention to avoid devastating consequences.

While perioperative AE presenting with macroglossia has been reported in cases of general anesthesia with endotracheal intubation, there are only a few case reports when a laryngeal mask airway (LMA) was used (11–13). It is important to note that with the application of an LMA

during surgery, additional mechanisms may contribute to development of macroglossia and airway compromise. An inappropriately selected size of the LMA (11) as well as patient positioning during surgery may facilitate formation of an edema and macroglossia which will be difficult to distinguish from AE affecting the tongue.

We present a clinical case of acute onset AE with macroglossia, which developed in the early post-operative period in a patient undergoing surgery in lateral position, when an LMA was used to secure the airway patency and provide ventilatory support. The patient had a history of chronic lisinopril treatment.

CASE DESCRIPTION

A 71 year old Caucasian male patient with a past medical history of coronary arterial disease, atrial flutter, ascending thoracic aortic dissection repair, aortic and mitral valve repair, 3-vessel coronary bypass, arterial hypertension, and bifascicular block was scheduled for an elective soft tissue biopsy of the ankle with possible resection and evacuation of the accumulated blood. He had no known history of allergies, complications of anesthesia, or any family history of angioedema. The list of home medications included: amlodipine, metoprolol, tylenol, apixaban, atorvastatin, lisinopril, spironolactone, tamsulosin, magnesium sulfate, and multivitamins. The patient weighed 90.7 kg and had a BMI of 26.39.

Following preoxygenation, anesthesia was induced with IV propofol 2 mg/kg and lidocaine 100 mg. A size 5 cuffed LMA was placed without any difficulty. The patient was then placed in right lateral position, and surgery was started. General anesthesia was maintained with inhalation of 0.8–0.9 age-adjusted minimum alveolar concentration of sevoflurane in a mixture of oxygen with air. Throughout surgery, the patient received 40 mg of ketamine in divided injections, and his blood pressure was supported with small boluses and low rate infusion of phenylephrine (0.2 mcg/kg/min). No antibiotics were administered during surgery. At the end of surgery, 8 mg of ondansetron was administered as an antiemetic. No narcotic analgesics were used, since the surgeon had used local anesthetic infiltration of the incision site during surgery.

The procedure was completed uneventfully with evacuation of collected blood and soft tissue biopsy, the LMA was removed, and the patient was transferred to the post-operative recovery unit fully awake and alert. A routine inspection of the oral cavity at the time of the LMA removal did not reveal any trauma to the mucosa or local edema.

In an hour after surgery, the anesthesiologist was notified by the recovery unit nurse that the patient complains on swelling of the tongue.

Immediate assessment revealed a progressively worsening swelling on the left half of the tongue without airway compromise. Hemodynamic and respiratory parameters remained within normal limits with SPO2 of 98%, heart rate 72/min, and normal blood pressure. In around an hour, the whole tongue became swollen, again with no signs of respiratory distress.

A preliminary diagnosis of AE was made considering the history of chronic lisinopril therapy. As alternative diagnoses, IgE-mediated anaphylactic reaction and macroglossia as a result of the LMA application and patient positioning were considered even though with lower possibility. Eight mg of dexamethasone and 25 mg of Benadryl were given IV, and inhalation of oxygen, 4 L/min was started. The otorhinolaryngology service was contacted to evaluate the patient, and emergency intubation equipment was made available. It was decided to observe the patient in the hospital setting overnight. An endoscopic examination performed by the otorhinolaryngologist revealed no signs of trauma or hematoma, the only finding was the observed isolated edema of the tongue without any airway compromise. A tryptase test was sent immediately, and the results came back normal: 4.3 mcg/L (normal values <11.0 mcg/L). The time period between the anesthesia induction and blood collection for the test was < 4 h. Empiric epinephrine was not used in our patient because of a history of significant cardiovascular disease and absence of any respiratory distress despite the lingual edema. The drug would be used as a first line choice in case of an apparent anaphylactic reaction with life-threatening airway compromise. A specific anti-bradykinin therapy with Icatibant was not used either, since the vital signs remained stable, and gradual improvement of the edema took place with time. Lastly, the drug has a relatively high cost, and its use in our patient with stable vital parameters and gradual resolution of symptoms would be questionable.

With conservative management and observation, the patient's condition improved with complete resolution of the edema within 24 h. No additional laboratory tests were ordered (measurement of C_1 estherase inhibitor activity and the C_4) at that time.

The patient was uneventfully discharged from the hospital next morning after an overnight observation. While in hospital, lisinopril therapy was discontinued, and the patient was referred to his primary care practitioner for further treatment and possible modifications in the medication plan.

DISCUSSION

AE is a symptom complex which may rarely complicate the perioperative period. In majority of cases, it remains self-limited and will only require supportive therapy with monitoring. However, in 11% of cases airway intervention is required to save the patient (1). In such patients, the mortality can be as high as 30–40% (3).

As mentioned above, three types of AE are known, and the treatment strategy will depend on etiologic mechanisms (3).

AE of allergic origin results from an allergic reaction of immediate type, when symptoms are caused by antigen-IgE mediated release of inflammatory mediators and histamine from the mast cells and basophils. In such cases, epinephrine, glucocorticoids, antihistamines (anti-H_1 and H_2 antagonists), and oxygen are usually effective. When indicated, bronchodilators and intravenous fluids should be administered.

Dexamethasone and Benadryl were administered to our patient, and inhalation of oxygen was initiated. Even though steroids and antihistamines are indicated only for AE of allergic origin, the possibility of acute deterioration of the respiratory function in the immediate post-operative period justified such an empiric therapy without a proof of allergic reaction. Epinephrine was not used considering adequate respiratory function during the whole period of observation and a history of significant cardiovascular disease. However, the plan was to use epinephrine should further worsening of swelling take place.

Drug-induced AE of non-allergic origin is commonly related to bradykinin overproduction or decreased metabolism. Bradykinin, increases microvascular permeability, promotes tissue edema, may induce arterial hypotension and bronchospasm. Most commonly, it is associated with the use of ACE inhibitors. The incidence of AE in patients receiving ACE inhibitors may reach 1.6%, and these drugs are believed to be the cause of drug-induced AE in 25–39% of cases (3, 4). AE in patients on such therapy may develop weeks or years after starting the treatment (4).

Other factors linked to bradykinin-mediated AE include treatment with angiotensin 2 receptor antagonists, non-steroidal anti-inflammatory drugs, latex allergy, surgical stress, and oropharyngeal instrumentation, including laryngoscopy (1). The principal therapy is discontinuation of the offending drug and oxygenation (9). Our patient had a history of chronic lisinopril therapy to control the arterial hypertension, and it is possible that lisinopril, along with surgical stress and placement of the LMA, could have contributed to development of AE.

Propofol is known to temporarily increase local bradykinin levels, which is the mechanism of pain at injection site. Even though the effect is transient, and the half-life of bradykinin is only 15 s (1), the capillary leak and edema may have a much longer duration. Propofol, in conjunction with surgical stress and mechanical manipulation during intubation, may contribute to edema formation in susceptible patients. Typically AE associated with propofol injection develops immediately following the injection (7). AE in our patient was noticed an hour following surgery, which practically excludes the possibility of AE development because of propofol injection.

Bradykinin-related AE is, in general, resistant to glucocorticoids and antihistamines. Fresh frozen plasma (FFP) has been suggested for treatment of bradykinin-induced AE, because it contains kinases accelerating bradykinin breakdown (3). However, there is a theoretical risk of AE exacerbation induced by C1 and kallikrein contained in FFP. There is some evidence that plasma pooled C1 inhibitor concentrate (C1-INH) may be effective in cases of drug-induced AE. In our case, such a treatment was not attempted, since the patient's clinical symptoms remained stable, and gradual improvement was observed.

AE of genetic origin in related to an inborn deficiency of decreased activity of C1-esterase inhibitor (1, 3, 4). These patients have a history of recurrent AE. Treatment options for hereditary AE include symptomatic management, watchful monitoring, and specific treatment with plasma pooled C1-INH (Berinert), specific kallikrein antagonist Ecallantide, and bradykinin B_2 receptor antagonist Icatibant (3). Antifibrinolytic drugs may be used, mostly prophylactically, to block the effects of plasmin on factor XIIa and reduce bradykinin production (3). Our patient did not have any history of hereditary AE, however, familiarity with this rare disorder is important for anesthesia providers.

An important goal for an acute onset AE involving the face, neck, and oropharynx is the maintenance of airway patency and adequate respiration. All patients with AE should be monitored for airway compromise, and prompt endotracheal intubation should be performed without hesitation if the edema progresses jeopardizing respiration. When indicated, a surgical airway must be considered.

In addition to AE, there are additional mechanisms that could have contributed to the macroglossia in our patient. It is possible that lateral positioning of the patient during surgery could have caused an uneven distribution of pressures around the LMA with resultant unequal lymphatic and venous drainage from the tongue. This could possibly result in unilateral macroglossia. There are a few literature reports of perioperative macroglossia, where similar factors were mentioned as a possible explanation for perioperative macroglossia. Stillman (11) describes development of macroglossia in an infant after using an inappropriately large LMA. Patient positioning during surgery can also predispose to development of macroglossia, which will be difficult to differentiate from AE (4, 8, 11–14). In such situations, mechanical trauma related to airway manipulation and impaired venous and lymphatic drainage play a role, and, usually macroglossia is evident immediately upon completion of surgery. Removal of the endotracheal tube or LMA relieves the pressure, improves drainage, and resolves the edema within a short period of time.

The clinical presentation in our patient was different: he was asymptomatic after surgery and developed progressive swelling of the tongue shortly after it. Thus, clinical presentation of our patient along with history of lisinopril therapy favors bradykinin-mediated AE as the primary diagnosis. AE caused by an allergic reaction is another possibility, since delayed drug-induced and latex related allergies have been reported. However, the tryptase test in our patient was normal, and no hypotension, flushing, bronchospasm or other typical allergic symptoms were present. Most of allergic reactions related to anesthetics take place immediately after administration of the drug, and delayed reactions are relatively rare. A complete allergological workup would be necessary, including a repeat tryptase test done at least 24 h after the reaction, to assess the peak to background ratio and, potentially, establish the cause of AE. However, our patient was discharged next morning with a referral to his primary care practitioner, and was lost to follow-up.

This is a re-demonstration of the well-known fact that in acute care, including perioperative period, oftentimes a final diagnosis cannot be established within a short time period, and symptomatic therapy to stabilize the patient becomes a priority.

The patient required an overnight observation to prevent an acute worsening of edema and airway compromise. All the necessary equipment for immediate airway control, should the situation worsen, was readily available. Fortunately, the patient's symptoms regressed with time, and he was discharged next

morning with complete resolution of edema. He was referred to his primary care practitioner for further therapy.

Icatibant, a selective, competitive B2 receptor antagonist, is recommended as an effective treatment option for bradykinin-induced AE. The drug has been approved for clinical use in the Unites States in 2019. Our patient did not receive Icatibant because of the following considerations: the patient's condition remained stable, and symptoms resolved within a day. Also, Icatibant should be used with caution in patients with cardiovascular disease (15). Lastly, the drug's high cost is a factor to consider for patients with stable clinical picture.

To our knowledge, this is the first reported case of a post-operative AE in a patient on chronic lisinopril therapy, who was operated on in lateral position with the use of an LMA. This patient case reminds of the possibility of AE in the perioperative period and highlights the importance of possible consequences. Extended monitoring, appropriate drug therapy and readiness to intervene to secure the airway if indicated is the recommended strategy. Specific therapy based on the type of AE, if known, should be used.

Our case report presents a single patient, and this is a limitation of this presentation. Because of the short duration of inpatient treatment and regression of symptoms with time, the diagnostic considerations were made on clinical judgement and limited available paraclinical data. However, AE is a rare phenomenon in perioperative setting, and even a single patient presentation will add to the current knowledge and help to better treat these patients.

This is a challenging category of patients, and focused studies are justified to develop evidence-based recommendations to manage AE in the perioperative period.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article and not covered by the patient safety and health privacy regulations will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SS provided anesthesia, performed a literature search, and wrote the manuscript. QF participated in patient management, searched literature, and wrote the manuscript. SB provided general supervision, participated in patient management, searched literature, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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The Effects of Cigarette Smoking on Steroidal Muscular Relaxants and Antibiotics Used: A Prospective Cohort Study

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Background: The impact of cigarette smoking on perianesthesia management is not clear elucidated. This paper studies the impact of long-term cigarette smoking on the dose-response of rocuronium and vecuronium used under general anesthesia and the type of antibiotics used after surgery.

Methods: We enrolled 240 participants from a teaching hospital in China in which finally enrolled in 221 participants. 106 participants have a history of long-term cigarette use and 115 participants without a history of smoking. All participants received general anesthesia for various surgeries, and rocuronium was used as the muscular relaxant. The primary outcome was the effective onset time of rocuronium after adjusting for its dose. The secondary outcomes included a recovery index and the time of muscle recovery changing from 25 to 75%.

Results: There was no measurable difference in the muscle relaxant onset time, duration of effectiveness, 75% recovery, recovery index, dose of opiates, anesthetics during surgery, or complication rate between smokers or non-smokers. However, the results showed a significant difference in antibiotic use between smokers and non-smokers (chi-squared = 13.695, $p < 0.001$), and a significant difference in the type of antibiotics used (chi-squared = 21.465, $p = 0.003$). Smokers had a significantly higher rate of cefathiamidine use.

Conclusion: Smoking cigarettes had no effect on muscle relaxants used under general anesthesia, but patients who had a history of smoking were more likely to receive antibiotics after surgery.

Clinical Trial Registration: http://www.chictr.org.cn/index.aspx, identifier ChiCTR-OIC-16009157.

Keywords: smoking-epidemiology, muscular relaxants, postoperative infection, general anesthesia, perioperation

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Abbreviations: BIS, bispectral index; BMI, body mass index; CI, confidence interval; EEG, electroencephalogram; NMBD, non-depolarizing neuromuscular blocking drug; OR, odds ratio; TOF, train of four; VT, tidal volume.

INTRODUCTION

The number of smokers has reached one billion globally, and China ranks first with over 300 million smokers. Smoking is a public health problem. It increases the incidence of adverse events and significantly affects postoperative recovery, these effects include addiction, priming for use of other addictive substances, reduced impulse control, deficits in attention and cognition, and mood disorders (Schmid et al., 2015). A number of reviews show that cigarette smoking is associated with increased peri-operative complications (Beckers and Camu, 1991; Rodrigo, 2000). It increases the risk of hospital mortality by 20% and the risk of major postoperative complications by 40% (Ruppert et al., 2018).

Rocuronium bromide is a non-depolarizing neuromuscular blocking drug (NMBD). It works by competitively binding to nicotinic acetylcholine receptors on motor endplates. It is intermediate-acting, rapid-onset, and works without cardiovascular side effects. It was introduced in clinical settings as a potentially ideal muscle relaxant (Özcengiz, 2005). The factors that affect onset time of muscle relaxants have been studied in recent two decades, and smoking was reported to be one of them (Dawson and Vestal, 1982). Nicotine in cigarettes is an alkaloid that has an agonist effect on nicotinic-cholinergic receptors. Small doses of nicotine (<100 μg/L) directly stimulate the neuromuscular junction (acetylcholine-like action) and facilitate transmission of impulses. Large doses (>100 μg/L) block transmission because of persistent depolarization (acute effect) or desensitization of the receptor site (chronic effect) (Taylor, 1992). Several clinical studies investigating the interactions between neuromuscular blocking agents and cigarette smoking have been reported, but whether smoking has substantial impact on neuromuscular blockade is controversial, as these studies have yielded contradictory results (Teiriä et al., 1996). We therefore launched a study investigating the possible effects of smoking on the onset time of rocuronium bromide through a prospective cohort study.

METHODS

Study Design

The study was approved by the Medical Ethics Committee of the Affiliated Hospital of Guizhou Medical University (Application Number: 2016-57) and was registered at the Chinese Clinical Trial Registry (Registration Number: ChiCTR-OIC-16009157). All the subjects gave informed consent before participating in the study.

From June 2016 to October 2018, we recruited patients undergoing surgery in the affiliated hospital of Guizhou medical university. All patients received general anesthesia for various surgeries. The inclusion criteria were patients over 18 years of age, volunteer participation in the study, and signed informed consent. The exclusion criteria were: a prior history of surgery; significant abnormal renal and liver function (AST 40 > U/L, ALT 50 > U/L, CRE > 73 μmol/L, or BUN > 7.5 mmol/L); disorders of neuromuscular junction and skeletal muscle; water and electrolyte disorder of extracellular fluid and intracellular fluid; taking medications that affect neuromuscular junction; or aggressive tumors.

Data Collection

We collected data at enrollment and each clinical encounter through standardized data collection forms, and the data were entered into electronic databases for analysis. Data collection included two parts: self-reported outcomes and intraoperative measurements. Self-reported outcomes included baseline information (including age, sex, etc.), as well as details of cigarette smoking and postoperative use of antibiotics.

Intraoperative Measurements

After the contraction response of electrical stimulation was stable, the calibration was completed, and the muscle relaxation was continuously monitored by four series of stimuli under the same conditions. The onset time for rocuronium was defined as the time from the end of the injection of rocuronium to the maximum depression of T1. The clinical action time was defined as the TOF ratio rising from 0 to 25%, and the recovery index was designated as the TOF ratio rising from 25 to 75%. The muscle relaxation monitoring concluded when the above three indicators were completed. The skin temperature tested in nasopharynges was monitored and maintained at not less than 32°C during the operation, and normothermia was also maintained throughout the procedure. After preoxygenated, sequential intravenous midazolam (0.05 mg/kg), 1% long-chain propofol (2 mg/kg), sufentanil (0.3 μg/kg), rocuronium (0.6 mg/kg) for anesthesia induction was conducted with mechanical ventilation after tracheal intubation, micro pump 1% medium long-chain propofol (6–8 mg/kgh), remifentanil (8–10 μg/kgh) after tracheal intubation, mechanical ventilation tidal volume (VT) (8–10 ml/kg), respiratory rate (12–16 times/min), to maintain an end-tidal carbon dioxide partial pressure of 35–45 mmHg. Anesthesia was maintained using intravenous and the BIS (bispectral index) of the EEG (electroencephalogram) was monitored to maintain a BIS value between 40 and 60.

Data Analysis

Preliminary analyses were performed to check the missing values in the dataset, fill in missing values through multiple imputations, categorize participants' ages into five levels (18–31 years, 31–40 years, 41–50 years, 51–60 years, and >60 years), categorize BMI (Body Mass Index) into three levels (<25 kg/m^2, 25–28 kg/m^2, and >28 kg/m^2), categorize the duration of smoking history into three levels (no smoke, <10 years, >10 years), categorize surgery types as major or minor, and transform postoperative complications into dichotomous variables (yes/no).

The first objective of the study was to detect differences between smokers and non-smokers in the effects of the muscle relaxant and in the postoperative use of antibiotics and opiates. Considering that the sample sizes of smokers and non-smokers were nearly equal, we used Fisher's exact test to determine whether significant differences between the two cohorts existed in categorical outcomes (categorized age; categorized BMI;

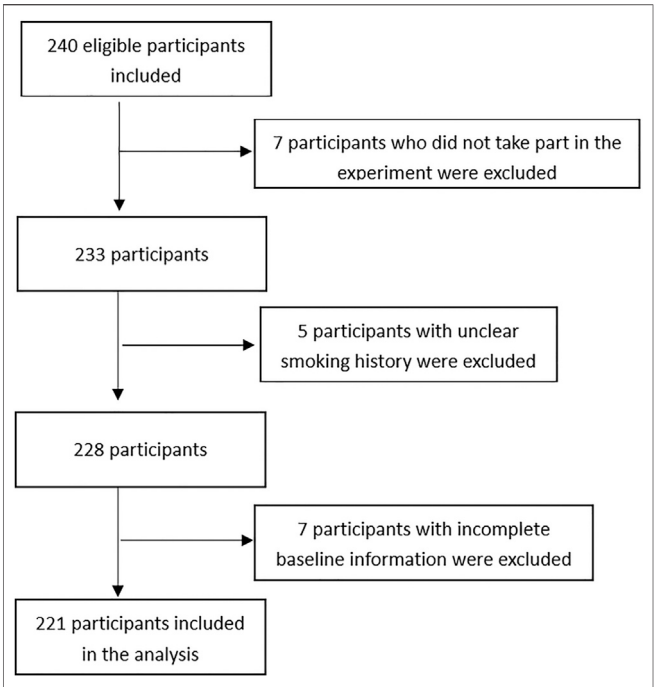

FIGURE 1 | Describes selection process of the study participants.

categorized smoke duration; proportion of patients quitting smoke; type of muscle relaxants; disease history; surgery type; usage of antibiotics; type of antibiotics, anesthetics, and opiates; and proportion of patients with complications). We also used independent *t* test to determine whether significant differences existed in continuous outcomes (PE_TCO2, onset time of muscle relaxants, lasting time of muscle relaxants, 75% recovery, and recovery index). We further used a logistic regression model to determine the robustness of Fisher's exact test and independent *t* test. We also studied the interaction between surgery type and the use of antibiotics, opiates, or anesthetics.

The second objective of the analysis was to determine the correlation of smoking frequency with use of muscle relaxant, antibiotics, opiates, and anesthetics. We used a linear regression model to find out whether the correlation was significant. We also used the model to find interaction between surgery type and the use of antibiotics, opiates, or anesthetics.

Coefficients of the logistic regression model and the linear regression model were calculated along with their corresponding 95% confidence intervals (95% CIs).

RESULTS

Characteristics of Study Participants

Figure 1 describes the selection process for the participants in the study. A total of 240 participants were screened for participation. Five were excluded for unclear history of cigarette smoking; seven were excluded for unwillingness to participate in the study; and seven were excluded for incomplete baseline information. The cohort study finally enrolled 221 participants. Characteristics of these participants at enrollment were summarized, and comparisons between the smokers and non-smokers were made in **Table 1**. The participants had an age of 44.29 ± 11.02 years and a BMI of 23.03 ± 2.84. Smokers had a mean smoke period of 10.86 ± 13.1 years. Two (1.9%) of the smokers had quit smoking prior to surgery. Thirty-three (14.93%) of the participants had a history of diabetes, hypertension, or hepatitis. The participants received 15 (6.87%) major surgeries and 206 (93.23%) minor surgeries. In the process of surgical procedure, participants were maintained at a mean PE_TCO2 of 36.07 ± 3.02, and they had a mean muscle relaxant onset time of 219.83 ± 60.04 s, a mean lasting effect of muscle relaxants of 2504.1 ± 799.22 s, a mean recovery index of 1165.91 ± 495.87 s, and a mean 75% recovery of 3672.84 ± 1015.99 s. A total of 138 (62.44%) participants were given postoperative antibiotic treatment. Five (2.26%) participants had postoperative complications.

Impact of Smoking on Use of Muscle Relaxants, Antibiotics, Anesthetics and Opiates

The result showed significant difference in antibiotic use between smokers and non-smokers (chi-squared = 13.695, $p < 0.001$) and significant difference in the type of antibiotics used (chi-squared = 21.465, $p = 0.003$). However, there was no difference in onset time of muscle relaxant, effective duration of musle relaxant, 75% recovery, recovery index, dosage of opiates and anesthetics during surgery, or complication rate between smokers or non-smokers. Logistic regression confirmed the significant difference in antibiotic use (odds ratio (OR) 2.87, 95% confidence interval (CI) 1.58 to 5.32; $p < 0.001$) between smokers and non-smokers, and showed that smokers had a significantly higher rate of cefathiamidine use (OR 7.92, 95% CI 1.50 to 62.48; $p = 0.023$). **Table 2** shows the ORs of variables in the logistic regression analysis. **Figure 2** shows the difference in onset time of muscle relaxant, effective duration of muscle relaxant, 75% recovery, recovery index, and dosage of opiates and anesthetics during surgery between smokers and non-smokers.

Impact of Smoking History Duration on use of Muscle Relaxants, Antibiotics, Anesthetics and Opiates

We also found that smoking history duration was significantly correlated with antibiotic use (estimate 4.98, 95% CI 2.00 to 7.97; $p = 0.001$) (**Table 3**). However, smoking history duration was not significantly correlated with onset time of muscle relaxant, effective duration of muscle relaxant, 75% recovery, recovery index, dosage of opiates and anesthetics during surgery, or complication rate. Further analysis showed that a longer history of smoking was correlated with higher rate of cefathiamidine use (estimate 7.92, 95% CI 0.38 to 16.40; $p = 0.04$).

TABLE 1 | Characteristics of included studies.

Variables	Smoking (*n* = 106)	Non-smoking (*n* = 115)	*p* value
Age			
18–30	7 (6.6)	6 (5.2)	0.3
31–40	8 (7.5)	19 (16.5)	
41–50	26 (24.5)	27 (23.5)	
51–60	41 (38.7)	34 (29.6)	
>60	24 (22.7)	29 (25.2)	
BMI			
<25	2 (1.9)	11 (9.6)	0.04
25–28	6 (5.7)	4 (3.5)	
>28	98 (92.4)	100 (86.9)	
Smoke period			
<10 years	5 (4.7)	—	
≥10 years	101 (95.3)	—	
Quit smoking			
Yes	2 (1.9)	—	
No	104 (98.1)	—	
Muscle relaxants			
Rocuronium	68 (64)	93 (81)	0.008
Vecuronium	38 (36)	22 (19)	
History			
Hypertension	7 (6.6)	13 (11.3)	0.5
Diabetes	4 (3.8)	2 (1.7)	
Both hypertension and diabetes	2 (1.9)	2 (1.7)	
Others	1 (0.7)	2 (1.7)	
None	92 (87)	96 (83.5)	
Surgery type			
Major	8 (7.5)	7 (6.1)	0.9
Minor	98 (92.5)	108 (93.9)	
PETCO2	35.7 (2.8)	36.4 (3.2)	0.1
Muscle relaxant onset time (s)	217.1 (55.9)	222.8 (64.4)	0.5
Muscle relaxant lasting effect time (s)	2420 (738.2)	2582 (847.3)	0.1
75% recovery	3631 (1003.4)	3711 (1030.3)	0.6
Recover index	1206 (509.3)	1129 (482.5)	0.2
Usage of antibiotics			
Yes	80 (75.5)	58 (50.4)	<0.001
No	26 (24.5)	57 (49.6)	
Type of antibiotics			
cefamandole	2 (1.9)	7 (6.1)	0.0
cefathiamidine	22 (21.0)	10 (8.7)	
cefoperazone	3 (2.9)	1 (0.9)	
ceftizoxime	6 (5.7)	5 (4.3)	
cefuroxime	28 (26.7)	22 (19.1)	
Levofloxacin	9 (8.6)	6 (5.2)	
Piperacillin	10 (9.5)	7 (6.1)	
None	26 (24.8)	57 (49.6)	
Anesthetics			
Propofol	95 (89.6)	91 (79.1)	0.05
Other	11 (10.4)	24 (20.9)	
Opiates			
Sufentanil	103 (97.1)	112 (97.4)	1
Fentanyl	3 (2.9)	3 (2.6)	
Complications			
Yes	4 (3.8)	1 (0.9)	0.3
No	102 (96.2)	114 (99.1)	

DISCUSSION

Our study found that cigarette smoking had no effect on the dosage of muscle relaxant during general anesthesia, and we found that cigarette smoking was associated with higher rate of antibiotic use postoperatively. One study showed that smokers appeared to require less atracurium than non-smokers (Kroeker et al., 1994). However, another study showed that smokers need more rocuronium to achieve the same muscle relaxation effect than non-smokers, and the investigators attributed this finding to high metabolic levels and changes in receptor levels in smokers (Rautoma and Svartling, 1998). These two studies showed contradictory results. Latorre and colleagues studied the effects of smoking on rocuronium in 20 smokers (Latorre et al., 1997).

TABLE 2 | Logistic regression analysis of the impact of cigarette smoking.

Variables	Coefficients	95% CI	*p* Value
Onset time	1.00	0.95 to 1.05	0.181
Lasting time	1.00	1.00 to 1.01	0.984
75% recovery	1.00	0.95 to 1.06	0.425
Recover index	1.00	0.95 to 1.05	0.975
Antibiotic use	2.87	1.58 to 5.32	0.960
Opiate dose	1.00	0.99 to 1.00	<0.001
Anesthetic dose	1.00	1.00 to 1.01	0.666
Complication	4.35	0.58 to 88.67	0.478
Cefathiamidine	7.92	1.50 to 62.48	0.024
Cefoperazone	9.08	0.68 to 266.53	0.124
Ceftizoxime	4.72	0.65 to 46.59	0.142
Cefuroxime	4.76	0.93 to 36.59	0.082
Levofloxacin	5.22	0.84 to 46.08	0.095
Piperacillin	4.67	0.80 to 39.49	0.109
Not use antibiotics	1.74	0.36 to 12.70	0.524

TABLE 3 | Linear regression analysis of the impact of cigarette smoking.

Variables	Coefficients	95% CI	*p* value
Onset time	−0.10	−0.32 to 0.11	0.349
Lasting time	0.00	−0.03 to 0.02	0.868
75% recovery	0.10	−0.11 to 0.32	0.354
Recover index	−0.10	−0.31 to 0.12	0.371
Antibiotic use	4.98	2.00 to 7.97	0.001*
Opiate dose	0.00	−0.03 to 0.02	0.722
Anesthetic dose	0.01	−0.03 to 0.04	0.694
Complication	4.67	−4.75 to 14.09	0.330
Cefathiamidine	7.92	0.38 to 16.40	0.040*
Cefoperazone	9.08	−4.25 to 20.90	0.193
Ceftizoxime	4.72	−6.25 to 12.98	0.491
Cefuroxime	4.76	−3.62 to 12.18	0.287
Levofloxacin	5.22	−5.92 to 11.92	0.508
Piperacillin	4.67	−3.82 to 13.36	0.275
Not use antibiotics	1.74	−7.55 to 7.43	0.988

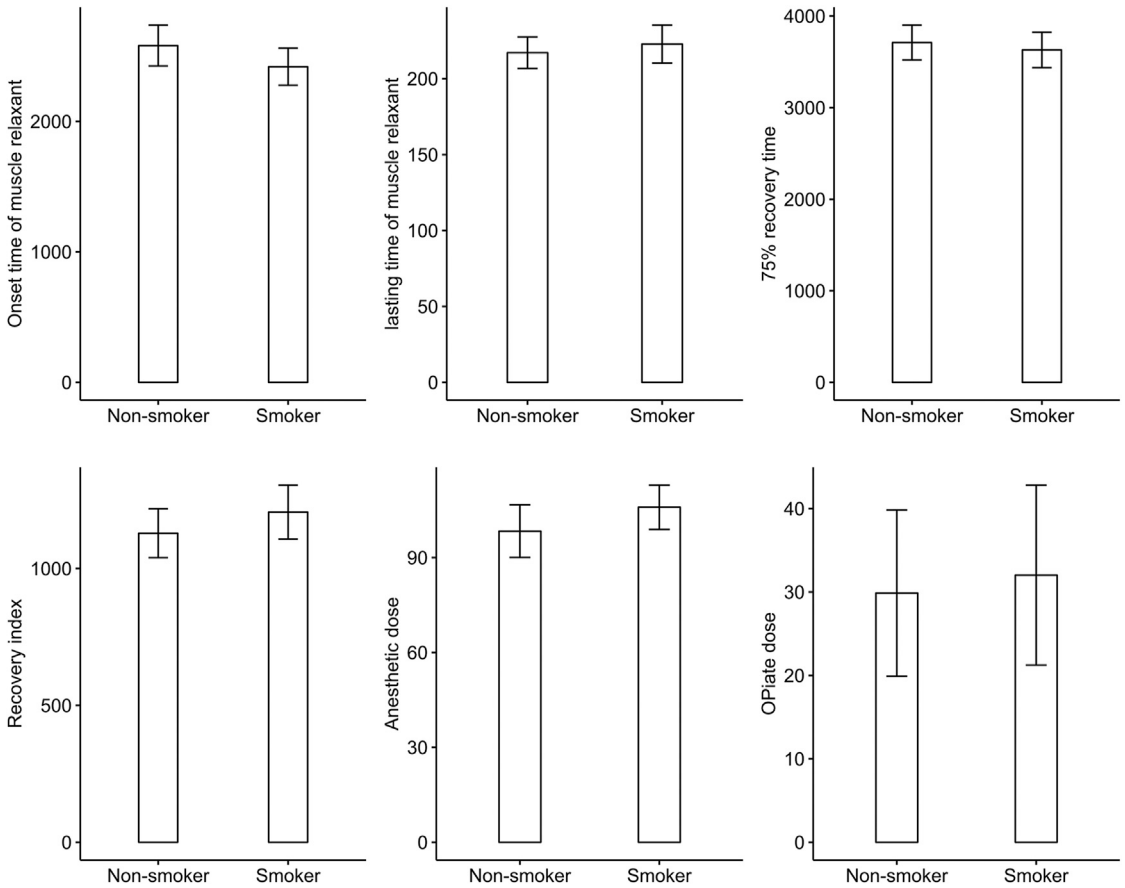

FIGURE 2 | Comparison in anesthesia related parameters between smokers and non-smokers.

They found that onset and recovery times were similar between smokers and nonsmokers, which could be partly due to a longer period of refraining from smoking in patients, leading to very low nicotine blood concentrations without the proposed receptor-stimulating effect. Friedrich and colleagues found similar outcomes, which showed that smoking did not change the dose-requirements for rocuronium and had no effects on the onset time, degree of blocking effect, time to maximum blocking effect, duration 10%, and spontaneous recovery index during anesthesia (Pühringer et al., 2000). The sample size of previous

studies was small (40 cases at most), which made it difficult to explain the problem clearly. In contrast, we determined the effect of long-term smoking on muscle relaxants under general anesthesia through large sample tests. In our study, duration of smoking history had no significant correlation with the onset time of muscle relaxant, the duration of the muscle relaxant's action, the 75% recovery rate, recovery index, the dosage of opioids and anesthetics during surgery, or the incidence of complications. This finding supports the viewpoint that smoking does not alter the dose-requirements for rocuronium and has no effects on the onset time (Latorre et al., 1997; Pühringer et al., 2000).

Literature showed that small doses of nicotine can increase the excitability of synapse acetylcholine, prolong the onset time, and weaken the effect of neuromuscular blockade. To the contrary, a large dose of nicotine would reduce the excitability of acetylcholine and would lead to the opposite results (Rodrigo, 2000; Sweeney and Grayling, 2009). However, the results of our study show that there is no difference between relaxation times and the fact of smoking.

Multiple studies have demonstrated that smoking increases the likelihood pulmonary complications (Bluman et al., 1998; Kaufmann et al., 2018). However, our results showed no difference between non-smokers and smokers. This could be explained by the fact that most of the procedures were minor surgeries, and the duration of operation was generally short. In addition, the proportion of antibiotics use was larger in smokers, which may have reduced the risk of postoperative complications.

We found significant difference in antibiotic use between smokers and non-smokers (chi-squared = 13.695, $p < 0.001$) and significant difference in the type of antibiotics used (chi-squared = 21.465, $p = 0.003$). This suggests that smoking patients might be more likely to suffer from postoperative infections and need more antibiotics than non-smokers. Active smoking is associated with respiratory complications, particularly bronchospasm and pneumonia (Ruppert et al., 2018). Meanwhile, the strong positive correlation between prevalence of COPD, emphysema, and lung fibrosis and prevalence of current smoking was expected among current and former smokers. Neuromuscular blockers as auxiliary drugs for general anesthesia are commonly used in tracheal intubation, which can cause respiratory muscle paralysis and affect respiratory function during and after surgery. These may also partly explain a higher rate of antibiotic use in participants with smoking habits. In addition, this finding may also suggest that using more antibiotics is an empirical choice that the doctor makes knowing that the patient is a smoker. More research is needed to clarify the mechanism of this finding.

Clindamycin and aminoglycoside antibiotics can enhance muscle relaxant function (Singh et al., 1978; Schulze et al., 1999). These antibiotics inhibit the release of acetylcholine from the motor nerve terminals by binding to calcium ions on the surface of presynaptic membranes (Pridgen, 1956). Therefore, doctors tend to use cephalosporin antibiotics without neuromuscular blocking effect, to avoid adverse neuromuscular blockade after surgery. For patients with a long history of smoking, prophylactic use of antibiotics before surgery may help to reduce the risk of pulmonary infection. However, further research needs to be done on the types of antibiotics, the timing of use, dosage, and course of treatment.

Our study had limitations. First, this study design was observational, so results should be treated with caution. We matched the smokers and non-smokers to reduce potential bias in the analysis of the primary outcome. Second, the smoking period and amount of cigarette use were based on the participants' self-report. Although we asked the participants to confirm their reports, the study was still under the risk of outcome assessment bias.

CONCLUSION

In summary, smoking does not alter the dose-requirements for rocuronium and has no effects on the onset time or the incidence of complications. It showed significant difference in antibiotic use between smokers and non-smokers and significant difference in the type of antibiotics used.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study was approved by the Affiliated Hospital of Guizhou Medical University. The approved protocol was registered. Each participant will be provided with information regarding the study. Written informed consent will be obtained from each patient. The privacy of all participants will be protected. Personal medical records will be reviewed by investigators, who will promise to keep the content confidential. It will be performed in accordance with the standards of the International Committee on Harmonization on Good Clinical Practice and the revised version of the Declaration of Helsinki principles.

AUTHOR CONTRIBUTIONS

NL performed the literature search and drafted the manuscript. XZ conducted a repeatability assessment of the study. FW, QZ, MC, and JS processed the data. XZ presented the concept and design of the research and revised the manuscript critically for important intellectual content.

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Intravenous Dexmedetomidine Administration Prior Anesthesia Induction With Propofol at 4°C Attenuates Propofol Injection Pain: A Double-Blind, Randomized, Placebo-Controlled Trial

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Background: Propofol injection pain, despite various interventions, still occurs during the anesthesia induction and causes intense discomfort and anxiety in patients. This study aimed to explore the effect of intravenous dexmedetomidine on propofol injection pain prior to anesthesia induction with propofol at 4°C.

Methods: A total of 251 patients (American Society of Anesthesiologists I–II) who underwent oral and maxillofacial surgery were randomly assigned to a combination group ($n = 63$), lidocaine group ($n = 62$), dexmedetomidine group ($n = 63$), and placebo-control group ($n = 63$); they received 0.5 ug/kg dexmedetomidine prior to anesthesia induction with propofol at 4°C, 40 mg lidocaine, 0.5 ug/kg dexmedetomidine prior to anesthesia induction, and normal saline, respectively. Incidence of pain, pain intensity, and reaction to the pain stimulus were evaluated by using verbal categorial scoring (VCS), a numerical rating scale (NRS), and the Surgical Pleth Index (SPI), respectively. In addition, hemodynamic parameters such as heart rate (HR) and mean arterial pressure (MAP) were also measured. The VCS and NRS were evaluated at 5 s after propofol injection. In addition, SPI, HR, and MAP were evaluated at three time points (before anesthesia induction and 5 and 30 s after propofol injection).

Results: The incidence of pain in the combination group (51%) was significantly lower than that in the lidocaine group (71%), dexmedetomidine group (67%), or placebo-control group (94%) ($p < 0.001$). VCS and NRS scores in the combination group were also lower compared with the other three groups ($p < 0.001$), with no statistically significant differences between the lidocaine group and dexmedetomidine group ($p > 0.05$). The SPI of the combination group decreased significantly in comparison with the other three groups at 5 s after propofol injection ($F = 96.23$, $p < 0.001$) and 30 s after propofol injection ($F = 4.46$, $p = 0.005$). Further comparisons between HR and MAP revealed no significant differences across the groups ($p > 0.05$).

Conclusion: Because of the sedative nature of dexmedetomidine and analgesic effect of low temperature, this study showed that intravenous dexmedetomidine prior to anesthesia induction with propofol at 4°C is highly effective in attenuating the incidence and severity of pain during injection compared with lidocaine (40 mg), dexmedetomidine 0.5 ug/kg) and placebo. This approach was not associated with any anesthesia complications.

Clinical Trial Registration: ClinicalTrials.gov, identifier: ChiCTR-2000034663

Keywords: dexmedetomidine, propofol, injection pain, lidocaine, cold temperature

INTRODUCTION

Propofol, a common intravenous anesthetic, is extensively used in induction, sedation, and maintenance of general anesthesia because of its rapid onset and quick patient recovery (1, 2). However, pain with its injection has been identified as a troubling experience for patients. About 28–90% of patients receiving propofol injection via the dorsal hand vein suffer different levels of pain intensity (3, 4). In addition, many anesthesiologists rank the pain experienced during propofol injection pain as the seventh-worst outcome among 33 known anesthesia outcomes, based on clinical importance and frequency (5). Injection pain may influence the quality of anesthesia in patients and cause an unpleasant experience (4, 6, 7).

Several factors account for propofol injection pain. For instance, increasing evidence has demonstrated that the lipid solvent irritates the vein intima and activates a local kallikrein-kinin cascade by releasing bradykinin and inflammatory factors (8, 9), and injection pain has been shown to occur when peripheral nerve endings are directly exposed to propofol (4). Moreover, investigations of clinical factors have revealed that female patients of younger age, with a peripheral vein site (especially the dorsum of hand), are highly sensitive to injection pain (10). Current recommendations suggest propofol injection in an antecubital vein for reducing propofol injection pain; however, this is not always practical in clinical situations.

Multiple strategies such as pharmacological and non-pharmacological therapy have proved to be efficacious in attenuating injection pain. In addition, drugs including lidocaine, ketamine, magnesium sulfate, and triglycerides are commonly used as pain relievers during propofol injection (4, 5, 11, 12). In a systematic review, it was concluded that lidocaine pretreatment effectively lowered the incidence of propofol injection pain (13). It is widely acknowledged that lidocaine is an acceptable anesthetic that prevents injection pain. Nevertheless, its adverse cardiovascular and hemodynamic effects and swelling at its injection site need to be further studied (14, 15). Recent reports have shown that dexmedetomidine (Dex-) is as highly effective in attenuating propofol-induced pain as lidocaine (6, 16). On the other hand, Dex- is a highly selective alpha-2 adrenoceptor agonist and exhibits analgesic, sedative, and sympatholytic properties that regulate the incidence and intensity of propofol injection pain. Non-pharmacological interventions such as the selection of larger veins (e.g., antecubital vein), adjusting injection speed, and controlling propofol dose (7, 17) are rarely in use for unknown reasons. Previous studies illustrated that temperature of propofol affected the intensity of injection pain and indicated that heating and cooling of propofol could lower pain intensity (18–20). Of note, propofol is generally kept at 25°C, and when warmed up to room temperature or 37°C, its chemical structure or efficiency may be altered. As a result, propofol at 4°C is more likely to be effective in reducing the intensity of pain.

Compared to lidocaine used alone, combination therapy is recommended in clinical situations (3). Therefore, the purpose of our study was to explore the effect of intravenous Dex- prior to anesthesia induction with propofol at 4°C in attenuating propofol injection pain compared with lidocaine applied independently.

MATERIALS AND METHODS

Study Design and Ethical Aspects

This study was designed as a double-blinded, randomized, and placebo-controlled clinical trial and all patients underwent oral and maxillofacial surgery at the Affiliated Stomatological Hospital of Sun Yat-sen University. The objectives and procedures of this study and adverse effects were explained completely, and all patients signed the written informed consent after a preoperative interview with an anesthetist. This clinical trial was conducted in accordance with the Declaration of Helsinki and obtained approval by the Ethics and Research Committee of the Affiliated Stomatological Hospital of Sun Yat-sen University, Guangzhou, China [Number (2018)3-105]. This trial was registered at the Chinese clinical trial registry (http://www.chictr.org.cn. Number ChiCTR-2000034663).

Study Participants

All patients were scheduled for elective surgery under general anesthesia between September 2018 and July 2019. The main exclusion criteria were as follows: patients aged <18 years, status of ASA > II, history of any drug allergies or use of analgesic medication within 24 h before surgery, any organ dysfunction, and neurological disease and mental disorders. A total of 278 patients were initially enrolled in this study; however, 16 patients were excluded after they declined to participate or surgery was canceled, 5 patients were also excluded as a result of the withdraw of surgery schedule, and 6 patients did not meet the inclusion

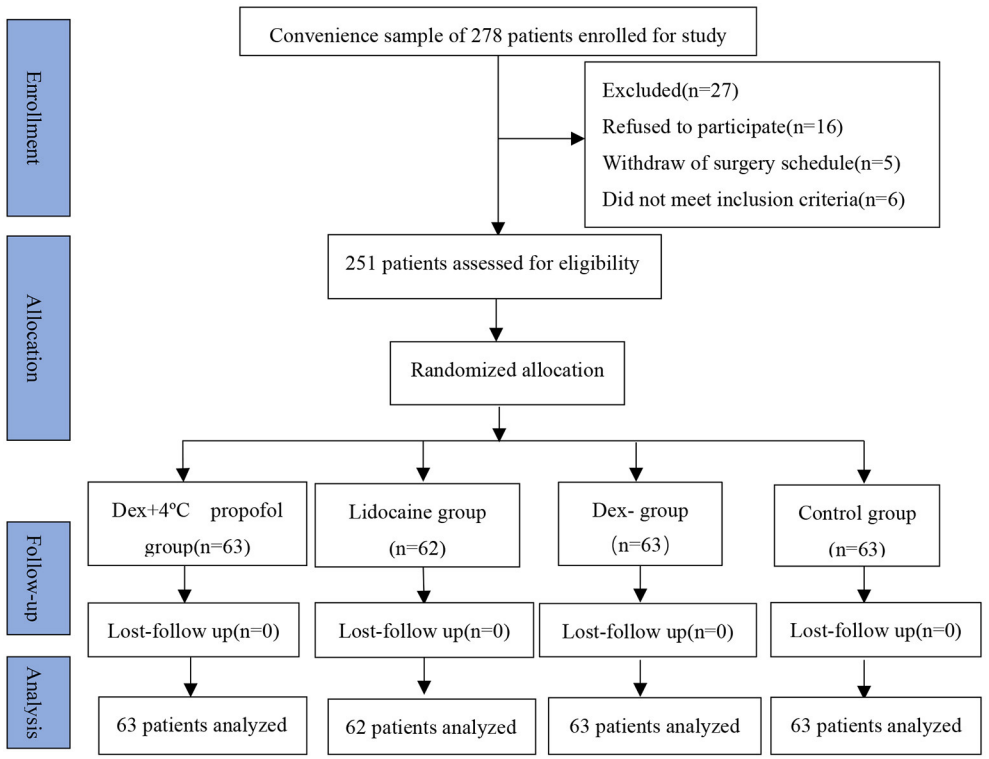

FIGURE 1 | CONSORT flow diagram of the study.

criteria. Eventually, 251 patients aged between 18 and 56 years with the American Society of Anesthesiologists physical status I–II (ASA I–II) were eventually enrolled in the four groups through random allocation (**Figure 1**).

Randomization and Blinding

Using a computer-generated randomization table, patients were randomly assigned into "Dex- (0.5 ug/kg) plus propofol at 4°C group (combination group)," "lidocaine group (40 mg)," "Dex- (0.5 ug/kg)," and "placebo-control group" according to a 1:1:1 ratio. A random allocation sequence of 251 consecutive and numbered envelopes, representing different group assignments, were directly delivered to an anesthetic nurse. Throughout the period of study and data collection, the principal investigator and participants remained unaware of each group assignment, while two anesthetic nurses and anesthetists were also blinded to participants' group assignment. the group assignments were announced only after finishing data analysis.

The Study Protocol and Data Collection

Demographic data including age, gender, American Society of Anesthesiologists (ASA) physical status, weight, and height were recorded for all patients in the 30 min before they entered the operating room. After entering the operating room, patients received standard monitoring of the electrocardiograph, non-invasive blood pressure, and pulse oximetry. Hemodynamic indexes, including mean arterial pressure (MAP) and heart rate (HR), were recorded when patients remained silent. After that, an 18G cannula was inserted into a large vein on the dorsum of the non-dominant hand 20 min before anesthesia induction, and an infusion of lactated Ringer's solution (5 ml/kg/h) was applied to maintain its patency.

Prior to anesthesia induction, Dex- (200 ug/2 ml, Hengrui Medicine, Jiangsu, China) was dissolved in a 50-ml syringe and the combination group was injected of 0.5 ug/kg DEX- for about 5 min, 40 mg lidocaine was dissolved in a 10-ml syringe and gently injected in 3 min in the lidocaine group, and patients in the placebo-control group were informed of being injected with an analgesic and received an equivalent volume of normal saline. Injections of Dex-, lidocaine, and normal saline were all undertaken via a three-way connector. Then, 6 L/min of pre-oxygenation was administered to patients with a mask, after which the principal anesthetist informed the anesthetic nurse to start anesthesia induction. During anesthesia induction, the combination group received propofol refrigerated at 4°C, and another three groups received propofol at room temperature. Because propofol injection (0.5 mg/kg) is a targeted dose, it should be injected within 30 s. However, it is worth noting that injection of 4°C propofol must be close to the puncture point of the intravenous infusion to avoid increasing its temperature. Therefore, another three-way connector close to the intravenous site was used to inject 4°C propofol.

In this experiment, primary outcomes included data collection on the pain scale of VCS, NRS, and SPI, and MAP and HR were

TABLE 1 | Baseline characteristics of included patients ($n = 251$).

Variables	Dex+4°Cpropofol ($n = 63$)	Lidocaine pre-administration ($n = 62$)	Dex- pre-administration ($n = 63$)	Control ($n = 63$)	F, χ^2	p
			Mean ± SD			
Age	31.32 ± 8.69	31.74 ± 7.97	32.76 ± 8.85	32.14 ± 9.62	0.33^F	0.81
Gender						
Female	34	31	31	28	$1.16^{\chi 2}$	0.76
Male	29	31	32	35		
ASA					$5.98^{\chi 2}$	0.11
I	53	49	47	57		
II	10	13	16	6		
Height(m)	1.68±0.08	1.67 ± 0.08	1.70 ± 0.08	1.66 ± 0.07	2.47^F	0.06
Weight(kg)	57.59 ± 10.02	60.11 ± 10.43	56.78 ± 9.19	59.06 ± 8.82	1.49^F	0.22
Heart rate(bpm)	75.81 ± 11.38	74.60 ± 10.80	74.33 ± 10.12	77.33 ± 12.29	0.94^F	0.42
Mean arteria pressure(mmHg)	84.59 ± 8.48	83.05 ± 8.44	82.76 ± 8.02	83.54 ± 8.27	0.59^F	0.62
Surgical pleth index (SPI)	69.27 ± 11.20	68.34 ± 9.78	69.90 ± 10.29	68.37 ± 11.21	1.25^H	0.74
Recovery time(sec)	285.84 ± 42.78	273.13 ± 42.14	282.48 ± 41.91	280.42 ± 36.73	3.02^H	0.39
Dose of propofol for anesthesia induction(mg)	115.17 ± 20.05	120.23 ± 20.86	113.56 ± 18.39	118.11 ± 17.64	4.02^H	0.26

F, ANOVA statistics; χ2, Chi-square test statistics; H, Kruskal-Wallis test statistics.

recorded as secondary outcomes. Two anesthetic nurses assessed the intensity of propofol injection pain in about 5–10 s by using VCS and NRS. VCS is a 4-point pain scale: 0–no pain, 1–mild pain with no behavioral sign, 2–moderate pain accompanied by a behavioral sign, and 3–severe pain with a strong vocal response accompanied by facial grimacing, arm withdrawal, or crying (7). Patients were instructed to report any discomfort through an 11-point NRS which ranges from 0 (no pain) to 10 (worst pain) (15). The arms of all patients were covered with a cloth to blind the two nurses to the group assignment. Moreover, a measure of the SPI was taken via an anesthesia monitor that recorded the patient's response to stressful surgical stimuli or pain during general anesthesia. MAP, HR, and SPI were separately recorded at three time points: before propofol injection, 5 s after propofol injection, and 30 s after propofol injection. After the targeted 0.5 mg/kg of propofol was finished, the remaining induction of propofol (1.5 mg/kg) at room temperature was administered immediately, accompanied by a complete injection of cis-atracurium (0.2 mg/kg) and remifentanil (1 ug/kg). After patients' eyelash reflexes began to disappear, a mixture of 100% oxygen and air combined with sevoflurane was delivered to the patient via a face mask. In the meantime, an anesthesiologist checked any anesthesia-induced complications after tracheal intubation. The dose of propofol for anesthesia induction, duration of surgery, and recovery time were recorded for the four groups.

Sample Size

Based on findings from a previous study in which 64% of patients experienced propofol injection pain (13), it was hypothesized that pre-administration of Dex- plus 4°C propofol would cause a 40% reduction in the injection pain. In our study, to provide the two-sided test at an α level of 0.05 and a power of 80%, we used a minimum sample size of 54 patients per group to detect a significant difference, considering a 15% dropout rate. We finally enrolled 63 patients in the combination group (0.5 ug/kg Dex- + 4°C propofol), 62 patients in the lidocaine group, 63 patients in the Dex- group and 63 patients in the placebo-control group (normal saline).

Statistical Analyses

All data were analyzed using SPSS 20.0 for Windows (SPSS, Inc, Chicago, IL, USA). Demographic and clinical characteristics were expressed as means ± standard deviation (continuous variables) or frequency (categorical variables) and analyzed by the χ^2-test and one-way ANOVA test across the groups. Because the VCS scores were not normally distributed, we used the Kruskal-Wallis test to compare VCS, whereas the Mann-Whitney U-test was used for multiple comparisons between different groups. Comparisons of the incidence of pain between groups were determined by the χ^2-test or Fisher's exact test. Repeated measures ANOVA was used to compare hemodynamic parameters (HR, MAP) between different groups and the three time points. A comparison of the two groups was made by Bonferroni correction where necessary. A $p < 0.05$ was considered statistically significant.

RESULTS

Demographic Characteristics

Demographic characteristics and clinical baseline data are summarized in **Table 1**. No significant differences were found between the four groups regarding age, gender, status of ASA, height, weight, HR, MAP, SPI, recovery time, and dose of propofol for anesthesia induction ($p > 0.05$).

TABLE 2 | Incidence and severity of propofol injection pain ($n = 251$).

Severity of pain(VCS)	Dex-+4°C propofol group ($n = 63$)	Lidocaine group ($n = 62$)	Dex- group ($n = 63$)	Control group ($n = 63$)
No pain (0)	31(49%)*#&	18(29%)&	21(33%)&	4(6%)
Mild pain (1)	29(46%)	35(56%)	29(46%)	31(49%)
Moderate pain (2)	3(5%)*#&	9(15%)&	13(21%)&	25(40%)
Severe pain (3)	0	0	0	3(5%)
Total Pain	32(51%)*#&	44(71%)&	42(67%)&	59(94%)

*$p < 0.001$; compared with lidocaine group.
#$p < 0.001$; compared with Dex- group.
&$p < 0.001$; compared with the control group.

Comparisons Between Groups in the Incidence and Severity of Propofol Injection Pain

The total incidence of propofol injection pain in the combination group (51%) was significantly lower than in the lidocaine group (71%), Dex- group (67%), or control group (94%) ($p < 0.001$) (**Table 2**). According to the different levels of severity of pain among the four groups, the incidence of patients with no pain in the combination group (49%) was the highest compared with the placebo-control group (6%), the lidocaine group (29%), and Dex- group (33%) ($p < 0.001$). Additionally, no significant differences in mild pain were found among the groups. However, the incidence of moderate pain in the placebo-control group (40%) was significantly higher compared to the other three groups ($p < 0.001$). Further, severe injection pain occurred in only three patients in the placebo-control group.

Comparisons Between Groups in the Scores of Pain VCS, Pain NRS, and SPI

By comparing the pain scores of VCS, NRS, and SPI between groups (**Table 3**), we found significant differences in VCS scores ($H = 44.90$, $p < 0.001$), Also, the NRS scores had statistically significant differences among the four groups ($F = 34.71$, $p < 0.001$). Meanwhile, it was obvious that scores of VCS and NRS in the combination group were also lower compared with the other three groups ($p < 0.05$). In other words, the combination group was superior to the other three groups with respect to pain reduction. Through ANOVA, SPI showed a significant difference among groups in 5 s after propofol injection ($F = 96.23$, $p < 0.001$) and 30 s after propofol injection ($F = 4.46$, $p = 0.005$). Primary outcomes in the combination group were significantly different from those in the other three groups, whereas the lidocaine group and Dex- group showed a significant difference compared with the placebo-control group.

Comparisons Between Groups in the HR and MAP

Comparisons of the hemodynamic parameters (HR, MAP) among the four groups are presented in **Table 4**. Repeated-measures ANOVA for hemodynamic parameters revealed a significant difference at different time points (HR, $F = 186.28$, $p < 0.001$), (MAP, $F = 182.23$, $p < 0.001$). Further, comparisons between HR and MAP showed no significant difference among the four groups (HR, $F = 1.24$, $p = 0.27$), (MAP, $F = 1.05$, $p = 0.31$), and interaction by times and groups was also showed no significant difference (HR, $F = 0.83$, $p = 0.37$), (MAP, $F = 0.69$, $p = 0.41$).

DISCUSSION

We designed a randomized, double-blind study to explore the efficacy of 0.5 ug/kg Dex- given prior to anesthesia induction with propofol at 4°C to attenuate injection pain in patients under general anesthesia. According to the findings, it was evident that 0.5 ug/kg Dex- pre-administration plus 4°C propofol had a favorable effect in alleviating propofol injection pain compared to lidocaine (40 mg) and Dex-(0.5 ug/kg). In addition, the difference between HR and MAP was not statistically significant among the four groups, and no anesthesia-induced complications such as anaphylaxis, hypotension, and bradycardia occurred during surgery.

Prevention of propofol injection pain deserves more attention, especially because of the discomfort experienced during anesthesia induction, and the mechanism of propofol injection pain should be elucidated. It is well-recognized that propofol is an excellent anesthetic that has a phenol group, and resultant pain can occur immediately or be delayed after propofol injection. However, propofol can directly irritate the afferent nerve endings within the mucous membranes and venous intima and cause immediate pain. Additionally, delayed pain arises when the kallikrein-kinin systems are activated by propofol molecules, generating bradykinin and inducing local vasodilation and hyperpermeability (6). Consequently, the contact between the aqueous phase of propofol and free nerve endings of vessel walls is increased (21). Nevertheless, a previous study revealed that the generation of bradykinin via activation of the plasma kallikrein-kinin system is completely not associated with propofol injection pain (22). It is worth noting that immediate pain caused by direct contact between propofol molecules and peripheral nerve endings is predominantly linked to propofol injection pain. As a result, we evaluated pain intensity in 5 s after injection of propofol by using VCS and NRS scales. Because propofol injection pain occurs rapidly once propofol injection starts, premedication for injection pain prior to anesthesia induction is a common approach in clinical practice.

TABLE 3 | Comparisons of pain scores (VCS/NRS) and surgical pleth index between groups ($n = 251$).

Variable	Time	Dex+4°C propofol ($n = 63$)	Lidocaine pre-administration ($n = 62$)	Dex- pre-administration ($n = 63$)	Control ($n = 63$)	F, χ^2	p
			Mean ± SD				
Scores of VCS	5 s after propofol injection	$0.56 \pm 0.59^{\#\&**}$	$0.85 \pm 0.65^{*}$	$0.87 \pm 0.73^{*}$	1.43 ± 0.69	44.67^{H}	<0.001
Scores of NRS	5 s after propofol injection	$2.86 \pm 1.35^{\#\&**}$	$3.61 \pm 1.70^{*}$	$3.57 \pm 1.96^{*}$	5.27 ± 1.88	21.72^{F}	<0.001
Surgical pleth index (SPI)	Prior to anesthesia induction	69.27 ± 11.20	68.34 ± 9.78	69.90 ± 10.29	68.37 ± 11.21	0.32^{F}	0.81
	5 s after propofol injection	$53.38 \pm 9.93^{\&**}$	$57.77 \pm 9.53^{\&*}$	$58.89 \pm 7.26^{\&*}$	77.60 ± 7.78	96.23^{F}	<0.001
	30s after propofol injection	$39.35 \pm 7.05^{\#\&*}$	43.37 ± 8.97	42.89 ± 8.17	44.32 ± 8.27	4.46^{F}	0.005

*$p < 0.05$; **$p < 0.001$; #: compared with lidocaine group; &, compared with Dex- group; $, compared with control group; F, ANOVA statistics; H, Kruskal-Wallis test statistics.

TABLE 4 | Comparisons of Hemodynamic parameters between groups ($n = 251$).

Variables	Time	Dex+4°C propofol ($n = 63$)	Lidocaine pre-administration ($n = 62$)	Dex- pre-administration ($n = 63$)	Control ($n = 63$)	*Source*	*F*	*p*
			Mean ± SD					
HR(bpm)	Prior to anesthesia induction	75.81 ± 11.38	74.60 ± 10.80	74.33 ± 10.12	77.33 ± 12.29	Time	186.28	<0.001
						Group	1.24	0.27
	5 s after propofol injection	76.16 ± 11.69	75.71 ± 8.77	75.29 ± 9.22	79.14 ± 11.73	T*G	0.83	0.37
	30 s after propofol injection	67.81 ± 7.95	66.60 ± 6.00	67.40 ± 7.99	68.75 ± 8.71			
MAP(bpm)	Prior to anesthesia induction	84.59 ± 8.48	83.05 ± 8.44	82.76 ± 8.02	83.54 ± 8.27	Time	182.33	<0.001
						Group	1.05	0.31
	5 s after propofol injection	80.19 ± 8.36	78.84 ± 8.57	78.83 ± 6.80	82.05 ± 8.51	T*G	0.69	0.41
	30 s after propofol injection	66.84 ± 7.38	68.24 ± 6.12	66.35 ± 6.45	69.14 ± 7.61			

HR, Heart rate; MAP, Mean arterial pressure; F, Repeated Measures ANOVA.

Because of its local anesthetic effects on the venous intima by inhibiting sodium-specific ion channels and stabilizing the kinin cascade (23), lidocaine pretreatment is widely used to produce a relative reduction in propofol injection pain. To achieve continuous effects with the use of lidocaine in reducing propofol injection pain, a tourniquet applied to the forearm is necessary to maintain its local analgesic effect by venous occlusion. However, the application time of the tourniquet should last about 30–60 s to exert a maximal effect, which unfortunately causes tourniquet-induced pain or discomfort, and the failure rate of lidocaine in pain relief ranged from 13 to 40% (18). In our study, we used a dose of 40 mg in the lidocaine group as previously recommended in a meta-analysis by Picard and Tramer (24). Given the discomfort caused by a tourniquet, lidocaine was slowly injected without a tourniquet in this trial. Nevertheless, a recent study used a mixture of lidocaine and propofol to pretreat injection pain, and it did not alleviate the propofol injection pain (25). In our study, the incidence of injection pain was reduced from 94% (control group) to 71% (40 mg lidocaine group), which suggested lidocaine pre-administration prior to anesthesia is effective in pain relief. However, the incidence of injection pain in the lidocaine group was still high, even though most patients experienced mild pain intensity.

Dex- may have a great impact on pain relief because of its significant sedative action. Dex-, as a highly potent alpha-2 adrenoreceptor agonist, has been proved to exert significant analgesic effects by raising the pain threshold and is also widely used in the sedation of critical patients on the basis of its sedative and anti-anxiety properties. The analgesic effect of Dex- could be achieved with a high dose of 1 ug/kg over 10 min (26); however, to avoid anesthetic complications, our study used just 0.5 ug/kg of Dex- pre-anesthesia, and its effect on pain relief possibly originates from its sedation property. Current evidence suggests that Dex- acts by inhibiting the release of substance P from the dorsal horn of the spinal cord and the spinal ERK1/2 signaling pathway (27–29). A study by Park et al. demonstrated that Dex- exerted a dose-dependent analgesic effect in rat models. Elsewhere, it was revealed that a high dose of Dex- in patients resulted in rapid pain relief (30, 31). Based on a previous report, a common dose of Dex- used in premedication of propofol injection pain was 0.25, 0.5, or 1 ug/kg; however, 0.25 ug/kg of Dex- did not effectively alleviate propofol injection pain (32). A recent study showed that the safety properties of Dex- at doses of 0.5 or 1 ug/kg could stabilize hemodynamics (33). Upon consultation with the anesthetist, we considered a 0.5 ug/kg dose of Dex- to be the most appropriate. Our study also indicated that

there was no statistically significant difference between Dex- and lidocaine in reducing propofol injection pain, and were similar to prior finding (6). Hence, the intravenous administration of Dex- appeared to be effective in pain relief as lidocaine.

Furthermore, low-temperature propofol generated contradictory outcomes in reducing injection pain. However, previous studies showed that using cold propofol in pain relief is an effective method because of decreased speed of the kinin cascade and the stabilization of local pain mediators (20, 34). On the contrary, findings from other studies have revealed that cold propofol had no impact on pain reduction (35), although pre-administration of 4°C propofol was recently shown to potentially lower the incidence of propofol injection pain from 70 to 30%. However, a systematic review demonstrated that 4°C propofol was not effective in reducing injection pain ($RR = 0.82$, 95% CI:0.64–1.04) (18). Therefore, a single use of 4°C propofol may not result in a remarkable analgesic effect. Therefore, we considered the analgesic effect of combining 0.5 ug/kg Dex- prior to the induction with propofol at 4°C. Notably, results showed that the incidence of injection pain was reduced from 94% (control group) to 51% (combination group), which explained the potential cumulative analgesic effect of 0.5 ug/kg Dex- in combination with propofol at 4°C.

There are some limitations to our study. First, we did not use a high dose of Dex-, such as 0.75 or 1 ug/kg, so it is not clear whether the effects of a high dose of dexmedetomidine could significantly reduce pain. Second, patients were not followed up for potential adverse effects after discharge of operation room. Finally, because patients in the lidocaine group did not use a tourniquet for venous occlusion, the contact between lidocaine and venous intima was not efficient. This explains why pain alleviation in the lidocaine group was inferior to that of the combination group.

CONCLUSION

In summary, considering the sedative nature of Dex- and the analgesic effect of low temperature, this double-blind, randomized, placebo-controlled trial demonstrated that intravenous Dex- prior to anesthesia induction with propofol at 4°C can effectively attenuate propofol injection pain compared with lidocaine (40 mg), Dex- (0.5 ug/kg), and placebo. It is worth noting that no significant adverse events arose during the intervention process.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://pan.baidu.com/s/1_tLnhB22hZVe4XbDPlHQSA~Code:7jrm&lt.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics and Research Committee of the Affiliated Stomatological Hospital of Sun Yat-sen University, Guangzhou, China. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YL, YG, and ZX conceived of the study and improved the design. YL and LL were responsible for collecting clinical data. XT and QX provided statistical analysis and interpretation. All authors approved the final manuscript.

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