



Mitochondria Are Potential Targets for the Development of New Drugs Against Neutrophilic Inflammation in Severe Pneumonia Including COVID-19

Nina V. Vorobjeva^{1*}, Galina F. Sud'ina² and Boris V. Chernyak²

¹Biology Faculty, M. V. Lomonosov Moscow State University, Moscow, Russia, ²A. N. Belozersky Institute of Physico-Chemical Biology, M. V. Lomonosov Moscow State University, Moscow, Russia

Keywords: pneumonia, COVID-19, neutrophils, mitochondria, reactive oxygen species, NADPH oxidase, neutrophil extracellular traps, NETosis

OPEN ACCESS

Edited by:

Ulrike Garscha,
University of Greifswald, Germany

Reviewed by:

Ewgenij Proschak,
Goethe University Frankfurt, Germany

*Correspondence:

Nina V. Vorobjeva
nvvorobjeva@mail.ru

Specialty section:

This article was submitted to
Inflammation Pharmacology,
a section of the journal
Frontiers in Pharmacology

Received: 23 September 2020

Accepted: 04 January 2021

Published: 29 January 2021

Citation:

Vorobjeva NV, Sud'ina GF and
Chernyak BV (2021) Mitochondria Are
Potential Targets for the Development
of New Drugs Against Neutrophilic
Inflammation in Severe Pneumonia
Including COVID-19.
Front. Pharmacol. 12:609508.
doi: 10.3389/fphar.2021.609508

INTRODUCTION: NEUTROPHIL HYPERACTIVATION IN INFLAMMATORY DISEASES

A high neutrophil-to-lymphocyte ratio is associated with disease severity and poor prognosis in pneumonia progressing to acute respiratory distress syndrome (ARDS) (Steinberg et al., 1994) including COVID-19 (Coronavirus Disease-19) caused by the novel SARS-CoV-2 coronavirus (Wang et al., 2020a; Mehta et al., 2020). Extensive infiltration of neutrophils into the pulmonary capillaries as well as their extravasation into the alveolar space have been described in bronchoalveolar lavage (Steinberg et al., 1994) and in autopsy specimens (Barnes et al., 2020; Wang et al., 2020b).

Neutrophils are the first line of defense against invading pathogens in the foci of inflammation, where they use effector functions such as phagocytosis, degranulation, and the formation of reactive oxygen species (ROS). Excessive activation of neutrophils results in the release of neutrophil extracellular traps (NETs) consisting of decondensed chromatin, «decorated» with myeloperoxidase (MPO), neutrophil elastase (NE), and other bactericidal proteins derived from intracellular granules (Brinkmann et al., 2004). The formation of NETs is usually accompanied by cell death, therefore, this process is called NETosis.

Examination of patients with severe pneumonia (Twaddell et al., 2019) as well as patients infected with SARS-CoV-2 (Zuo et al., 2020) revealed an increased level of NETosis markers, such as cell-free DNA, MPO-DNA-complexes, citrullinated histone H3, and a marker of cell death lactate dehydrogenase. In the serum from patients with COVID-19 the concentration of cell-free DNA correlated with the content of neutrophils, the marker of the acute phase of inflammation C-reactive protein, and the marker of thrombosis D-dimer (Zuo et al., 2020). This serum induced NETosis in healthy donor blood in an *in vitro* system (Barnes et al., 2020; Zuo et al., 2020). One of the manifestations of COVID-19 is Kawasaki syndrome, a vasculitis that occurs in children and is accompanied by excessive NETosis (Yoshida et al., 2020). NETosis in COVID-19 can be caused by epithelial and endothelial cells affected with the virus, by activated platelets, and by inflammatory cytokines. At the same time, excessive NETosis is involved in the development of the «cytokine storm» and immunothrombosis, which are the main cause of severe complications associated with COVID-19 (Wang et al., 2020a; Barnes et al., 2020), as well as with H1N1 influenza and some other viral infections (Cantan et al., 2019).

THE ROLE OF MITOCHONDRIA IN NEUTROPHIL ACTIVATION

When activated, leukocytes accumulating in the lungs cause damage to the capillary endothelium and alveolar epithelium. Destruction of blood vessel endothelial cells can lead to blood coagulation and strokes (Ackermann et al., 2020). The attachment of neutrophils is mediated by adhesion molecules ICAM1, VCAM, etc., exposed on the surface of endothelial cells. The expression of these molecules is induced by inflammatory cytokines through the activation of the transcription factor NF- κ B. We recently found that the expression of adhesion molecules is dependent on the production of mitochondrial ROS (mtROS), which contribute to NF- κ B activation (Zinovkin et al., 2014; Galkin et al., 2016; Zakharova et al., 2017). Moreover, mtROS are critical for the increase in endothelium permeability and endothelial cell apoptosis induced by the inflammatory cytokine TNF (Galkin et al., 2016). The mitochondria-targeted antioxidants SkQ1 (10-(6'-methylplastoquinonyl) Decyltriphenylphosphonium) and SkQR1 (10-(6'-plastoquinonyl) decylrhodamine 19) protect endothelial cells *in vitro* and prevent increased expression of adhesion molecules and the lethal effect of TNF in a mouse model of systemic inflammatory syndrome (Zakharova et al., 2017). The mechanism of increased production of mtROS in the endothelium is not clear, but in the related model of endothelial stimulation with angiotensin II this mechanism has been shown to depend on mitochondrial permeability transition pore opening (mPTP) (Itani et al., 2016). Inhibitors of the mPTP are currently being developed and studied as potential drugs against cardiovascular diseases (Briston et al., 2019). It is possible that these drugs, when combined with mitochondria-targeted antioxidants, could be effective in preventing endothelial damage in a variety of inflammatory pathologies including pneumonia and COVID-19.

Our recent studies have shown that mtROS play a key role in neutrophil activation (Vorobjeva et al., 2017; Vorobjeva et al., 2020). The mitochondria-targeted antioxidant SkQ1 inhibited degranulation and ROS production induced by the chemoattractant fMLP via G-protein coupled receptor. It was concluded that mtROS are involved in the assembly and activation of the multicomponent enzyme complex NADPH oxidase (NOX2), which is the main source of ROS in neutrophils. The same cross-talk between mtROS and NADPH oxidase has been described in the endothelial cells (Nazarewicz et al., 2013). As in the endothelium, mtROS production in human neutrophils depends on the opening of mPTP (Vorobjeva et al., 2020). One of the important consequences of neutrophil activation is a delay in spontaneous apoptosis. This effect was blocked by mitochondria-targeted antioxidants (Vorobjeva et al., 2017), so it is possible that these agents could not only inhibit the damaging activity of neutrophils, but also reduce their number in inflammatory lesions.

Our experiments with the mitochondria-targeted antioxidant SkQ1 showed its potential efficacy against NETosis (Vorobjeva et al., 2020). It was shown that NETs formation induced by Ca^{2+} ionophore A23187 depends on the mPTP opening and the

generation of mtROS. NETosis in this model was mediated by mtROS-dependent activation of NADPH oxidase. The massive production of ROS by NADPH oxidase, in turn, stimulated the opening of mPTP and mtROS generation. We have demonstrated that SkQ1 interrupted this vicious circle effectively neutralizing mtROS (Vorobjeva et al., 2017; Vorobjeva et al., 2020). The antioxidant MitoQ, which is structurally similar to SkQ1, suppressed NETosis in the mouse model of systemic lupus erythematosus (Fortner et al., 2020).

THERAPEUTIC POTENTIAL OF TARGETING NEUTROPHIL HYPERACTIVATION IN SEVERE PNEUMONIA INCLUDING COVID-19

Various therapeutic approaches have been developed to inhibit neutrophils accumulation in the foci of inflammation, their activation and NETosis, as well as factors destroying NETs. They include anticytokine therapy directed against IL-1 β (Anakinra; a recombinant IL-1 β receptor antagonist is currently in clinical trials against COVID-19; <https://clinicaltrials.gov: NCT04324021, NCT04330638, NCT02735707>. 2020.), and NETosis may be one of its targets. Low molecular weight IL-8/CXCR2 antagonists have been tested in clinical trials for asthma, chronic obstructive pulmonary disease (COPD) and influenza, and have shown suppression of pulmonary neutrophilia and a decrease in the signs of NETosis (Narasaraju et al., 2020), but they have not been tested in COVID-19 trials. Among NETosis inhibitors, especially extensive research and testing has been conducted with NE inhibitors. The first of these, Sivelestat, has been approved for use against ARDS in Japan and South Korea, but meta-analysis of clinical data did not confirm its effectiveness (Tagami et al., 2014). New generation NE inhibitors have been clinically tested for the treatment of COPD and may hold promise for COVID-19, as well as recombinant DNase I that degrades NETs (Narasaraju et al., 2020). The *in vitro* experiments have shown that NETosis can be prevented with the microtubule inhibitors. This group of drugs includes the oldest anti-inflammatory drug Colchicine, which, despite its strong cytotoxicity, is successfully used in small doses to treat acute gout and some other inflammatory diseases (Leung et al., 2015). Clinical trials of the efficacy of Colchicine against COVID-19 are currently underway (<https://clinicaltrials.gov: NCT04326790, NCT04328480, NCT04322565, NCT04322682.2020>).

Leukotrienes, metabolites of arachidonic acid produced in the 5-lipoxygenase (5-LOX) pathway, are lipid mediators of inflammation involved in asthma and COPD, which are also considered potential targets for COVID-19 therapy (Funk and Ardakani, 2020). The 5-LOX pathway is activated in many diseases and triggers inflammatory responses that are not affected by glucocorticoids. Leukotriene B₄, produced from leukotriene A₄ by the soluble leukotriene A₄ hydrolase, is a potent neutrophil chemoattractant that mediates the airway neutrophilia seen in severe COVID-19 (Wang et al., 2020b; Barnes et al., 2020). Another metabolite of arachidonic acid, epoxy fatty acids (EpFA), exhibits anti-inflammatory properties

in contrast to leukotrienes. Prevention of NF- κ B activation by EpFA can be mediated by inhibition of mPTP and subsequent production of mtROS (Wagner et al., 2020). To promote the accumulation of EpFA, inhibitors of soluble epoxide hydrolase (sEH), which converts EpFA to less active metabolites, have been developed (Wagner et al., 2020). An excellent effect in the resolution of inflammation was achieved by the double inhibition of leukotriene A4 hydrolase and sEH (Hefke et al., 2020; Hiesinger et al., 2020). Dual 5-LOX/sEH inhibition significantly suppressed leukocyte activation (Meirer et al., 2016), and neutrophil infiltration (Garscha et al., 2017). Another approach to the treatment of COVID-19, based on the use of the cysteinyl leukotriene receptor 1 (CysLT1) antagonist Montelukast, was recently proposed (<https://clinicaltrials.gov/ct2/show/NCT04389411>). In a small group of elderly patients with asthma, the use of Montelukast reduced the risk of SARS-CoV-2 infection (Bozek and Winterstein, 2020).

REFERENCES

- Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., et al. (2020). Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N. Engl. J. Med.* 383, 120–128. doi:10.1056/NEJMoa2015432
- Barnes, B. J., Adrover, J. M., Baxter-Stoltzfus, A., Borczuk, A., Cools-Lartigue, J., Crawford, J. M., et al. (2020). Targeting potential drivers of COVID-19: neutrophil extracellular traps. *J. Exp. Med.* 217, e20200652. doi:10.1084/jem.20200652
- Bozek, A., and Winterstein, J. (2020). Montelukast's ability to fight COVID-19 infection. *J. Asthma*, 1–2. doi:10.1080/02770903.2020.1786112
- Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D. S., et al. (2004). Neutrophil extracellular traps kill bacteria. *Science* 303, 1532–1535. doi:10.1126/science.1092385
- Briston, T., Selwood, D. L., Szabadkai, G., and Duchon, M. R. (2019). Mitochondrial permeability transition: a molecular lesion with multiple drug targets. *Trends Pharmacol. Sci.* 40, 50–70. doi:10.1016/j.tips.2018.11.004
- Cantan, B., Luyt, C. E., and Martin-Loeches, I. (2019). Influenza infections and emergent viral infections in intensive care unit. *Semin. Respir. Crit. Care Med.* 40, 488–497. doi:10.1055/s-0039-1693497
- Fortner, K. A., Blanco, L. P., Buskiewicz, I., Huang, N., Gibson, P. C., Cook, D. L., et al. (2020). Targeting mitochondrial oxidative stress with MitoQ reduces NET formation and kidney disease in lupus-prone MRL-lpr mice. *Lupus Sci. Med.* 7, e000387. doi:10.1136/lupus-2020-000387
- Funk, C. D., and Ardakani, A. (2020). A novel strategy to mitigate the hyperinflammatory response to COVID-19 by targeting leukotrienes. *Front. Pharmacol.* 11, 1214. doi:10.3389/fphar.2020.01214
- Galkin, I. I., Pletjushkina, O. Y., Zinovkin, R. A., Zakharova, V. V., Chernyak, B. V., and Popova, E. N. (2016). Mitochondria-targeted antioxidant SkQR1 reduces TNF-induced endothelial permeability *in vitro*. *Biochemistry Mosc.* 81, 1188–1197. doi:10.1134/S0006297916100163
- Garscha, U., Romp, E., Pace, S., Rossi, A., Temml, V., Schuster, D., et al. (2017). Pharmacological profile and efficiency *in vivo* of diflupolol, the first dual inhibitor of 5-lipoxygenase-activating protein and soluble epoxide hydrolase. *Sci. Rep.* 7, 9398. doi:10.1038/s41598-017-09795-w
- Hefke, L., Hiesinger, K., Zhu, W. F., Kramer, J. S., and Proschak, E. (2020). Computer-aided fragment growing strategies to design dual inhibitors of soluble epoxide hydrolase and LTA4 hydrolase. *ACS Med. Chem. Lett.* 11, 1244–1249. doi:10.1021/acsmchemlett.0c00102
- Hiesinger, K., Schott, A., Kramer, J. S., Blöcher, R., Witt, F., Wittmann, S. K., et al. (2020). Design of dual inhibitors of soluble epoxide hydrolase and LTA₄ hydrolase. *ACS Med. Chem. Lett.* 11, 298–302. doi:10.1021/acsmchemlett.9b00330
- Itani, H. A., Dikalova, A. E., McMaster, W. G., Nazarewicz, R. R., Bikineyeva, A. T., Harrison, D. G., et al. (2016). Mitochondrial cyclophilin D in vascular oxidative

CONCLUSION AND FUTURE PROSPECT

We hypothesize that mitochondria-targeted antioxidants and mPTP inhibitors may have beneficial effects in patients with severe COVID-19. These agents can be used alone or in the combination with other drugs. For example, the antioxidant N-acetylcysteine has recently been shown to enhance the action of Sivelestat against inflammatory pathology (Raevens et al., 2020). In summary, mitochondria appear to be a promising target for further drug development against severe pneumonia including COVID-19.

AUTHOR CONTRIBUTIONS

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

- stress and hypertension. *Hypertension* 67, 1218–1227. doi:10.1161/HYPERTENSIONAHA.115.07085
- Leung, Y. Y., Yao Hui, L. L., and Kraus, V. B. (2015). Colchicine--update on mechanisms of action and therapeutic uses. *Semin. Arthritis Rheum.* 45, 341–350. doi:10.1016/j.semarthrit.2015.06.013
- Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., Manson, J. J., et al. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 395, 1033–1034. doi:10.1016/S0140-6736(20)30628-0
- Meirer, K., Glatzel, D., Kretschmer, S., Wittmann, S. K., Hartmann, M., Blöcher, R., et al. (2016). Design, synthesis and cellular characterization of a dual inhibitor of 5-Lipoxygenase and soluble epoxide hydrolase. *Molecules* 22, 45. doi:10.3390/molecules22010045
- Narasaraju, T., Tang, B. M., Herrmann, M., Muller, S., Chow, V., and Radic, M. (2020). Neutrophilia and NETopathy as key pathologic drivers of progressive lung impairment in patients with COVID-19. *Front. Pharmacol.* 11, 870. doi:10.3389/fphar.2020.00870
- Nazarewicz, R. R., Dikalova, A. E., Bikineyeva, A., and Dikalov, S. I. (2013). Nox2 as a potential target of mitochondrial superoxide and its role in endothelial oxidative stress. *Am. J. Physiol. Heart Circ. Physiol.* 305, H1131–H1140. doi:10.1152/ajpheart.00063.2013
- Raevens, S., Van Campenhout, S., Debacker, P. J., Lefere, S., Verhelst, X., Geerts, A., et al. (2020). Combination of sivelestat and N-acetylcysteine alleviates the inflammatory response and exceeds standard treatment for acetaminophen-induced liver injury. *J. Leukoc. Biol.* 107, 341–355. doi:10.1002/JLB.5A1119-279R
- Steinberg, K. P., Milberg, J. A., Martin, T. R., Maunder, R. J., Cockrill, B. A., and Hudson, L. D. (1994). Evolution of bronchoalveolar cell populations in the adult respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* 150, 113–122. doi:10.1164/ajrccm.150.1.8025736
- Tagami, T., Tosa, R., Omura, M., Fukushima, H., Kaneko, T., Endo, T., et al. (2014). Effect of a selective neutrophil elastase inhibitor on mortality and ventilator-free days in patients with increased extravascular lung water: a post hoc analysis of the PiCCO pulmonary edema study. *J. Intensive Care* 2, 67. doi:10.1186/s40560-014-0067-y
- Twaddell, S. H., Baines, K. J., Grainge, C., and Gibson, P. G. (2019). The emerging role of neutrophil extracellular traps in respiratory disease. *Chest* 156, 774–782. doi:10.1016/j.chest.2019.06.012
- Vorobjeva, N., Galkin, I., Pletjushkina, O., Golyshev, S., Zinovkin, R., Prikhodko, A., et al. (2020). Mitochondrial permeability transition pore is involved in oxidative burst and NETosis of human neutrophils. *Biochim. Biophys. Acta (BBA) - Mol. Basis Dis.* 1866, 165664. doi:10.1016/j.bbdis.2020.165664
- Vorobjeva, N., Prikhodko, A., Galkin, I., Pletjushkina, O., Zinovkin, R., Sud'ina, G., et al. (2017). Mitochondrial reactive oxygen species are involved in chemoattractant-induced oxidative burst and degranulation of human neutrophils *in vitro*. *Eur. J. Cell. Biol.* 96, 254–265. doi:10.1016/j.ejcb.2017.03.003

- Wagner, K. M., Gomes, A., McReynolds, C. B., and Hammock, B. D. (2020). Soluble epoxide hydrolase regulation of lipid mediators limits pain. *Neurotherapeutics* 17, 900–916. doi:10.1007/s13311-020-00916-4
- Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., et al. (2020b). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *J. Am. Med. Assoc.* 323, 1061–1069. doi:10.1001/jama.2020.1585
- Wang, J., Jiang, M., Chen, X., and Montaner, L. J. (2020a). Cytokine storm and leukocyte changes in mild versus severe SARS-CoV-2 infection: review of 3939 COVID-19 patients in China and emerging pathogenesis and therapy concepts. *J. Leukoc. Biol.* 108, 17–41. doi:10.1002/JLB.3COVR0520-272R
- Yoshida, Y., Takeshita, S., Kawamura, Y., Kanai, T., Tsujita, Y., and Nonoyama, S. (2020). Enhanced formation of neutrophil extracellular traps in Kawasaki disease. *Pediatr. Res.* 87, 998–1004. doi:10.1038/s41390-019-0710-3
- Zakharova, V. V., Pletjushkina, O. Y., Galkin, I. I., Zinovkin, R. A., Chernyak, B. V., Krysko, D. V., et al. (2017). Low concentration of uncouplers of oxidative phosphorylation decreases the TNF-induced endothelial permeability and lethality in mice. *Biochim. Biophys. Acta (BBA) - Mol. Basis Dis.* 1863, 968–977. doi:10.1016/j.bbadis.2017.01.024
- Zinovkin, R. A., Romaschenko, V. P., Galkin, I. I., Zakharova, V. V., Pletjushkina, O. Y., Chernyak, B. V., et al. (2014). Role of mitochondrial reactive oxygen species in age-related inflammatory activation of endothelium. *Aging* 6, 661–674. doi:10.18632/aging.100685
- Zuo, Y., Yalavarthi, S., Shi, H., Gockman, K., Zuo, M., Madison, J. A., et al. (2020). Neutrophil extracellular traps in COVID-19. *JCI insight* 5, e138999. doi:10.1172/jci.insight

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

Copyright © 2021 Vorobjeva, Sud'ina and Chernyak. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.