COMMENTARY

Journal of
Clinical Pharmacy and Therapeutics | WILEY

Neutrophil elastase inhibitor (sivelestat) may be a promising therapeutic option for management of acute lung injury/acute respiratory distress syndrome or disseminated intravascular coagulation in COVID-19

Adeleh Sahebnasagh PharmD, BCPS[1] | Fatemeh Saghafi PharmD, BCPS[2] | Mohammadreza Safdari MD[3] | Masoud Khataminia PharmD, BCPS[4] | Afsaneh Sadremomtaz PhD[5] | Zeinab Talaei PhD[6] | Hassan Rezai Ghaleno MD[7] | Mahdi Bagheri PharmD, BCPS[8] | Solomon Habtemariam PhD[9] | Razieh Avan PharmD, BCPS[10] (iD)

[1]Department of Internal Medicine, Clinical Research Center, Faculty of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran

[2]Department of Clinical Pharmacy, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

[3]Department of Orthopedic Surgery, Faculty of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran

[4]Student Research Committee, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

[5]XB20 Drug Design, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, The Netherlands

[6]Department of Chemistry, Faculty of Science, Shahid Rajaee Teacher Training University, Tehran, Iran

[7]Department of Surgery, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

[8]Baqiyatallah Hospital, Baqiyatallah University of Medical Sciences, Tehran, Iran

[9]Pharmacognosy Research Laboratories and Herbal Analysis Services, School of Science, University of Greenwich, Kent, UK

[10]Department of Clinical Pharmacy, Medical Toxicology and Drug Abuse Research Center (MTDRC), Faculty of Pharmacy, Birjand University of Medical Sciences, Birjand, Iran

Correspondence
Razieh Avan, Department of Clinical Pharmacy, Medical Toxicology and Drug Abuse Research Center (MTDRC), Faculty of Pharmacy, Birjand University of Medical Sciences, Birjand, Iran.
Email: avanr91@gmail.com

Abstract

What is known and objective: This article summarizes the effects of sivelestat on acute lung injury/acute respiratory distress syndrome (ALI/ARDS) or ARDS with co-agulopathy, both of which are frequently seen in patients with COVID-19.

Comment: COVID-19 patients are more susceptible to thromboembolic events, including disseminated intravascular coagulation (DIC). Various studies have emphasized the role of neutrophil elastase (NE) in the development of DIC in patients with ARDS and sepsis. It has been shown that NE inhibition by sivelestat mitigates ALI through amelioration of injuries in alveolar epithelium and vascular endothelium, as well as reversing the neutrophil-mediated increased vascular permeability.

What is new and conclusions: Sivelestat, a selective NE inhibitor, has not been evaluated for its possible therapeutic effects against SARS-CoV-2 infection. Based on its promising beneficial effects in underlying complications of COVID-19, sivelestat

could be considered as a promising modality for better management of COVID-19–induced ALI/ARDS or coagulopathy.

KEYWORDS

acute lung injury/acute respiratory distress syndrome, coagulopathy, COVID-19, neutrophil elastase inhibito, sivelestat

1 | WHAT IS KNOWN AND OBJECTIVE

The little-known respiratory infection by a novel coronavirus 2019-nCoV in December 2019 has now become a global pandemic. The virus and the disease it causes are officially designated as SARS-CoV-2 and COVID-19, respectively.[1] At the time of preparing this manuscript, WHO reported over ten million confirmed positive cases of COVID-19 and the number of global deaths now exceeds 499 913 (https://covid19.who.int/?gclid=CjwKCAjwxev3BRBBEiwA iB_PWDVmEMC7Tn5vgLxqlWMio-sKDYOMlfZ0Z-u7CEdDsriO CxOop6cf2hoCOHoQAvD_BwE). The death rate of COVID-19 is estimated to be 2%,[2] which is less than SARS and the Middle East respiratory syndrome (MERS), but SARS-CoV-2 has proven to be more contagious.[3]

Although the symptoms of COVID-19 vary depending on age and underlying conditions,[4] most patients exhibit symptoms such as fever, dry cough, myalgia, tiredness, and diarrhoea. Patients with COVID-19 are also more susceptible to thromboembolic events because of immobility, inflammation, hypoxia and disseminated intravascular coagulation (DIC). In one study, the incidence of thrombotic complications in patients admitted to the intensive care unit (ICU) with COVID-19 was reported to reach 31%.[5] In this regard, hyperfibrinolysis related to increased levels of D-dimer was observed in 97% of patients with COVID-19 at the time of hospital admission and it continued to rise in all patients before death. Fibrin degradation products also significantly increased during the course of the disease. In severe cases of COVID-19 or in dying patients, a significant drop in platelet levels was observed.[6] Numerous reports outlined that a severe form of the disease in COVID-19 patients develops during a few days, which is often manifested as an acute lung injury/acute respiratory distress syndrome (ALI/ARDS), respiratory failure, heart failure or sepsis.[4] The animal models of SARS-CoV and MERS-CoV showed that the significant levels of inflammatory and immune responses cause 'cytokine storm' and apoptosis of epithelial and endothelial cells. This is followed by an increase in vascular permeability and leakage, abnormal T-cell and macrophage responses, and ALI/ARDS that could eventually lead to death.[7]

In patients with COVID-19, the inflammatory cytokine storm is closely associated with the development and progression of ARDS.[8] In these patients, the high-level cytokine expression of interleukin (IL)-1β, interferon (IFN)-γ–induced protein (IP-10) and monocyte chemoattractant protein 1 (MCP-1) could result in activated T-helper-1 (Th1) response. When compared to hospitalized COVID-19 patients on general wards, those in the ICU appear to show a higher level of granulocyte colony-stimulating factor, IP-10, MCP-1, macrophage inflammatory protein-1A, and tumour necrosis factor-alpha (TNF-α). Several studies emphasized this direct correlation between cytokine storm and the severity of COVID-19.[9] Furthermore, an elevated level of IL-6 was shown to be a predictor of poor outcome in severe COVID-19 with pneumonia and ARDS.[10] Hence, the inflammatory cytokine storm plays a key role both for the development of ARDS and extra-pulmonary organ failure.[8]

Acute respiratory distress syndrome is a severe type of acute lung injury and is characterized by massive infiltration of neutrophils, monocytes and lymphocytes. The diffuse bilateral oedema followed by reduced lung compliance, alveolar damage and bronchoalveolar lumen hyaline deposition result in hypoxic respiratory failure. Degranulating neutrophils have a key role in the development of capillary injury and leakage and hyaline deposition. These events may progress to ARDS or a more fatally diffuse alveolar damage.[11] The key role of neutrophils in the pathogenesis of ALI/ARDS has also been shown in animal and clinical studies. Histological assay on autopsy samples of ARDS patients has illustrated a significant accumulation of polymorphonuclear cells (PMN) including neutrophils in the damaged alveoli and the interstitial tissues.[12]

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a basic transcription factor that is essential for the expression of inflammation-related genes such as inducible nitric oxide synthase (iNOS) and inflammatory cytokines.[13] In patients with ARDS, the activation of NF-κB leads to increased expression of immunoregulatory and pro-inflammatory cytokines.[14]

Viral infection has also the potential to induce the production of oxidized products or oxidative stress that aggravates the inflammation-mediated COVID-19 pathology. For example, oxidized low-density lipoprotein under SARS-induced ALI activates the innate immune response. This leads to the overproduction of IL-6 in alveolar macrophages via the Toll-like receptor 4 (TLR4)/NF-κB signalling pathway.[15] Furthermore, with an active viral infection, the retinoic acid–inducible gene I (RIG-I) senses viral RNA and triggers signalling cascades, adaptor proteins (MAVS and TRAF) and different transcription factors (NF-κB and IRF3/IRF7) at host pattern recognition receptors (PRRs). This accounts for the initiation of antiviral type I interferon transcription and pro-inflammatory cytokines.[16]

As major components of inflammatory responses to endothelial injury, neutrophils have proteolytic and pro-apoptotic properties

through the action of several enzymes.[17] Among them is the serine protease, neutrophil elastase (NE), which has antimicrobial properties due to its ability to degrade phagocytosed pathogens.[18] It also contributes to inflammation by increasing vascular permeability[19] and induction of pro-inflammatory cytokines release, such as IL-6 and IL-8.[20] In this regard, NE is needed for neutrophil function during sepsis. Under normal physiological conditions, the function of NE is rigorously regulated by endogenous protease inhibitors.[20] However, under exaggerated inflammatory conditions, NE is enabled to attack the endothelial barrier and infiltrate to bronchoalveolar space. This is due to the inactivation of protease inhibitors by neutrophil oxidants.[21] Thus, excessive activity of NE may lead to tissue damage and remodelling in several pulmonary diseases such as community-acquired pneumonia, ventilator-associated pneumonia, exacerbated COPD, cystic fibrosis, bronchiectasis and ALI/ARDS.[22] Furthermore, in those patients with ALI/ARDS, the plasma levels of NE are significantly higher in comparison with healthy subjects.[23] Significantly higher level of proteolytic activity of NE was also observed in the bronchoalveolar lavage (BAL) of ARDS patients.[24]

Sivelestat, also known in the scientific literature as ONO-5046, is a selective, reversible and competitive neutrophil elastase inhibitor. Hence, it does not affect the function of other proteases in the body.[25] Its protective effects in attenuating ALI/ARDS have been described in several models of lung injury. In different preclinical and animal models of lung injury, sivelestat mitigated the lung vascular permeability, elevated pulmonary artery pressure (PAP), lung tissue wet to dry weight ratio, and neutrophil count.[26-28] Furthermore, sivelestat improved pathogen clearance, the decrease in PaO_2, and prevented digestion of surfactant protein D.[29,30]

2 | COMMENT

The following databases were searched to identify relevant literature concerning empirical evidence: The Cochrane Library, PubMed, MEDLINE and EMBASE from 1980 through March 2020.

It has been shown that NE inhibition by sivelestat mitigates ALI through amelioration of injuries in alveolar epithelium and vascular endothelium, as well as reversing neutrophil-mediated increased vascular permeability.[26] In a clinical study on patients with ARDS and systemic inflammatory response syndrome, continuous infusion of sivelestat has been shown to significantly improve pulmonary function, as indicated by an increase in PaO2/FIO2 ratio, shortened duration of mechanical ventilation time and length of ICU care. However, the mortality rate did not differ in comparison with the control group.[31] In another phase III clinical double-blinded trial with 230 ALI patients, sivelestat was shown to increase recovery rates of the pulmonary functional parameters, reduce the duration of the mechanical ventilator and better weaning rates, and shortened ICU length of stay by almost half. However, this selective NE inhibitor was not able to reduce the 30-day survival rate. In this study, only

around 20% of death occurred due to respiratory failure. This may explain why the survival rate between the two arms of the study did not reach a significant value.[32]

Several studies have indicated that sivelestat increases ventilator-free days and survival in patients with ALI/ARDS through inhibition of the overstretch-induced signalling pathway and neutrophil chemotaxis.[33-35] During mechanical ventilation (MV), the risk of ALI is increased due to over-activation of neutrophil elastase and myeloperoxidase. Furthermore, phosphorylation of c-Jun NH2-terminal kinase (JNK) is increased in alveolar type 2 epithelial cells.[36] There is a direct relationship between the inhibition of JNK and the prevention of over-ventilation lung injury. JNK is also critical in the induction of apoptosis during stress responses.[37] The protective effects of sivelestat against MV were evaluated in an animal model of mice where sivelestat (100 mg/kg, intraperitoneally) or saline was administered 30 minute before ventilation. Under 4 hours of MV with a high tidal volume of 20 mL/kg, sivelestat prevented histopathological MV-induced lung damage, decreased lung tissue wet to dry weight ratio, and suppressed the serum and bronchoalveolar lavage fluid levels of macrophage-inflammatory protein 2 (MIP-2), IL-6 and TNF-α. Through inhibition of neutrophil chemotaxis, sivelestat also normalized the phosphorylation of JNK and attenuated apoptotic changes in pneumocytes after the MV-induced ALI.[38]

In a retrospective study of sivelestat in 110 patients with ALI and sepsis, sivelestat significantly increased the number of ventilator-free days and PaO2/FIO2, especially in those patients with baseline procalcitonin levels of ≥0.5 ng/mL.[35] In another multicenter, prospective study using 164 mechanically ventilated ARDS patients with high wet to dry lung weight ratio, the efficacy of sivelestat was compared to the control group. In this study, sivelestat increased ventilator-free days with no significant effect on 28-day mortality.[34]

In a phase IV open-label, non-randomized, multi-centre clinical trial for the treatment of ALI associated with SIRS on 581 patients, sivelestat was continuously administered intravenously at a dose of 0.2 mg/kg/h for 2 weeks. The results of this study showed a significantly higher ventilator-free day, ICU discharge rate and early weaning from the mechanical ventilator with sivelestat compared to the control group. Furthermore, those patients received sivelestat had a substantially higher 180-day survival than control patients. The results of this clinical trial emphasized on the clinical efficacy of this NE inhibitor in this group of patients.[39]

During ARDS and cytokine storm, the host's inflammatory responses, including serum levels of TNF-α, IL-6, high mobility group box 1 (HMGB1) protein and NO synthesis from iNOS, rise significantly.[40-42] As indicated above, the NF-κB signalling pathway regulates the expression of these inflammatory factors.[43,44] Furthermore, NE-mediated chemotaxis to the lung potently induces epithelial cells and macrophages to produce inflammatory cytokines.[45] It has been demonstrated that sivelestat downregulates the NF-κB pathway and inhibits the secretion of HMGB1 from macrophages through inhibition of IκB kinase phosphorylation.[46] Moreover, following the administration of sivelestat, the serum levels of TNF-α, IL-6, HMGB1 and NO were shown to

decrease significantly. This selective NE inhibitor could also substantially reduce the level of MCP-1 mRNA in macrophages during ischemia-reperfusion injury.[47]

As outlined above, COVID-19 patients are more susceptible to thromboembolic events. In this regard, various studies have emphasized on the role of NE in the development of disseminated intravascular coagulation (DIC) in patients with ARDS and sepsis. In a study on 167 septic patients with ARDS and DIC, sivelestat was administered upon admission to ICU and continued for 5 days. The results showed that sivelestat improved lung injury score, PaO_2/FIO_2 ratio, DIC score, and ICU length of stay and survival rate when compared to the control group.[48]

In another study on 142 ARDS patients with DIC, the efficacy of sivelestat alone, recombinant human soluble thrombomodulin (rhTM) alone, combination therapy of sivelestat and rhTM or untreated control were evaluated and compared to each other. The results were very promising, indicating that combination therapy with sivelestat and rhTM significantly increased the 60-day patient survival, mechanical ventilator-free days, better PIO_2/FIO_2 ratio and DIC score.[49] Recently, neutrophil elastase inhibitors were proposed as a potential prophylactic treatment option for COVID-19 patients.[50]

3 | WHAT IS NEW AND CONCLUSIONS

An overview of the effects of sivelestat on ALI/ARDS or ARDS with coagulopathy was presented, both of which are frequently seen in patients with COVID-19. Sivelestat has not been evaluated for its possible therapeutic effects against COVID-19. Nevertheless, based on its promising beneficial effects in underlying complications of COVID-19, this selective NE inhibitor could be considered as a promising treatment for better management of ALI/ARDS or coagulopathy in patients with COVID-19. We believe that testing the possible efficacy of this therapeutic modality in this group of patients could help in developing new strategies for combating COVID-19.

CONFLICT OF INTEREST
None declared.

ORCID
Razieh Avan https://orcid.org/0000-0002-6412-5596

REFERENCES
1. Lai C-C, Shih T-P, Ko W-C, Tang H-J, Hsueh P-R. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and corona virus disease-2019 (COVID-19): the epidemic and the challenges. *Int J Antimicrob Agents*. 2020;55(3):105924.
2. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med*. 2020;8(4):420-422.
3. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *JAMA*. 2020;323(11):1061-1069.
4. Zhang R, Wang X, Ni L, et al. COVID-19: melatonin as a potential adjuvant treatment. *Life Sci*. 2020;250:117583.
5. Klok F, Kruip M, Van der Meer N, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res*. 2020;191:145–147.
6. Ji H-L, Zhao R, Matalon S, Matthay MA. Elevated plasmin (ogen) as a common risk factor for COVID-19 susceptibility. *Physiol Rev*. 2020;100(3):1065-1075.
7. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin Immunopathol*. 2017;39(5):529-539.
8. Ye Q, Wang B, Mao J. Cytokine storm in COVID-19 and treatment. *J Infect*. 2020.80:607–613.
9. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506.
10. Cao X. COVID-19: immunopathology and its implications for therapy. *Nat Rev Immunol*. 2020;20(5):269-270.
11. Kast R. Dapsone as treatment adjunct in ARDS. *Exp Lung Res*. 2020;46(5):157-161.
12. Kambas K, Markiewski MM, Pneumatikos IA, et al. C5a and TNF-α up-regulate the expression of tissue factor in intra-alveolar neutrophils of patients with the acute respiratory distress syndrome. *J Immunol*. 2008;180(11):7368-7375.
13. Singleton KD, Beckey VE, Wischmeyer PE. Glutamine prevents activation of NF-κB and stress kinase pathways, attenuates inflammatory cytokine release, and prevents acute respiratory distress syndrome (ARDS) following sepsis. *Shock*. 2005;24(6):583-589.
14. Moine P, McIntyre R, Schwartz MD, et al. NF-κB regulatory mechanisms in alveolar macrophages from patients with acute respiratory distress syndrome. *Shock*. 2000;13(2):85-91.
15. Imai Y, Kuba K, Neely GG, et al. Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. *Cell*. 2008;133(2):235-249.
16. Li Z, Li X, Huang Y-Y, et al. FEP-based screening prompts drug repositioning against COVID-19. *bioRxiv*. 2020.
17. Aikawa N, Kawasaki Y. Clinical utility of the neutrophil elastase inhibitor sivelestat for the treatment of acute respiratory distress syndrome. *Ther Clin Risk Manag*. 2014;10:621.
18. Segal AW. How neutrophils kill microbes. *Annu Rev Immunol*. 2005;23:197-223.
19. Ma Y, Yang X, Chatterjee V, Meegan JE, Beard RS Jr, Yuan SY. Role of neutrophil extracellular traps and vesicles in regulating vascular endothelial permeability. *Front Immunol*. 2019;10:1037.
20. Kawabata K, Hagio T, Matsuoka S. The role of neutrophil elastase in acute lung injury. *Eur J Pharmacol*. 2002;451(1):1-10.
21. Sandhaus RA, Turino G. Neutrophil elastase-mediated lung disease. *COPD*. 2013;10(sup1):60-63.
22. Polverino E, Rosales-Mayor E, Dale GE, Dembowsky K, Torres A. The role of neutrophil elastase inhibitors in lung diseases. *Chest*. 2017;152(2):249-262.
23. Kodama T, Yukioka H, Kato T, Kato N, Hato F, Kitagawa S. Neutrophil elastase as a predicting factor for development of acute lung injury. *Intern Med*. 2007;46(11):699-704.
24. Mcguire WW, Spragg RG, Cohen AB, Cochrane CG. Studies on the pathogenesis of the adult respiratory distress syndrome. *J Clin Investig*. 1982;69(3):543-553.
25. Pu S, Wang D, Liu D, et al. Effect of sivelestat sodium in patients with acute lung injury or acute respiratory distress syndrome: a meta-analysis of randomized controlled trials. *BMC Pulm Med*. 2017;17(1):148.
26. Miyazaki Y, Inoue T, Kyi M, Sawada M, Miyake S, Yoshizawa Y. Effects of a neutrophil elastase inhibitor (ONO-5046) on acute pulmonary injury induced by tumor necrosis factor Alpha (TNF α) and activated neutrophils in isolated perfused rabbit lungs. *Am J Respir Crit Care Med*. 1998;157(1):89-94.

27. Kubo K, Kobayashi T, Hayano T, et al. Effects of ONO-5046, a specific neutrophil elastase inhibitor, on endotoxin-induced lung injury in sheep. *J Appl Physiol*. 1994;77(3):1333-1340.

28. Sakamaki F, Ishizaka A, Urano T, et al. Effect of a specific neutrophil elastase inhibitor, ONO-5046, on endotoxin-induced acute lung injury. *Am J Respir Crit Care Med*. 1996;153(1):391-397.

29. Hagio T, Matsumoto S, Nakao S, Abiru T, Ohno H, Kawabata K. Elastase inhibition reduced death associated with acid aspiration-induced lung injury in hamsters. *Eur J Pharmacol*. 2004;488(1–3):173-180.

30. Hagio T, Kishikawa K, Kawabata K, et al. Inhibition of neutrophil elastase reduces lung injury and bacterial count in hamsters. *Pulm Pharmacol Ther*. 2008;21(6):884-891.

31. Okayama N, Kakihana Y, Setoguchi D, et al. Clinical effects of a neutrophil elastase inhibitor, sivelestat, in patients with acute respiratory distress syndrome. *J Anesth*. 2006;20(1):6-10.

32. Tamakuma S, Ogawa M, Aikawa N, et al. Relationship between neutrophil elastase and acute lung injury in humans. *Pulm Pharmacol Ther*. 2004;17(5):271-279.

33. Hashimoto S, Okayama Y, Shime N, et al. Neutrophil elastase activity in acute lung injury and respiratory distress syndrome. *Respirology*. 2008;13(4):581-584.

34. Tagami T, Tosa R, Omura M, et al. 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*. 2014;2(1):1-8.

35. Miyoshi S, Hamada H, Ito R, et al. Usefulness of a selective neutrophil elastase inhibitor, sivelestat, in acute lung injury patients with sepsis. *Drug Des Devel Ther*. 2013;7:305.

36. Uhlig S. Ventilation-induced lung injury and mechanotransduction: stretching it too far? *Am J Physiol Lung Cell Mol Physiol*. 2002;282(5):L892-L896.

37. Janssen-Heininger YM, Persinger RL, Korn SH, et al. Reactive nitrogen species and cell signaling: implications for death or survival of lung epithelium. *Am J Respir Crit Care Med*. 2002;166(12 Pt 2):S9-S16.

38. Sakashita A, Nishimura Y, Nishiuma T, et al. Neutrophil elastase inhibitor (sivelestat) attenuates subsequent ventilator-induced lung injury in mice. *Eur J Pharmacol*. 2007;571(1):62-71.

39. Aikawa N, Ishizaka A, Hirasawa H, et al. Reevaluation of the efficacy and safety of the neutrophil elastase inhibitor, Sivelestat, for the treatment of acute lung injury associated with systemic inflammatory response syndrome; a phase IV study. *Pulm Pharmacol Ther*. 2011;24(5):549-554.

40. Bhatia M, Moochhala S. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *J Pathol*. 2004;202(2):145-156.

41. Medzhitov R, Janeway CA Jr. Decoding the patterns of self and nonself by the innate immune system. *Science (New York, NY)*. 2002;296(5566):298-300.

42. van der Poll T, van Deventer SJ. Cytokines and anticytokines in the pathogenesis of sepsis. *Infect Dis Clin North Am*. 1999;13(2):413-426.

43. Liu SF, Malik AB. NF-kappa B activation as a pathological mechanism of septic shock and inflammation. *Am J Physiol Lung Cell Mol Physiol*. 2006;290(4):L622-L645.

44. Blackwell TS, Christman JW. The role of nuclear factor-kappa B in cytokine gene regulation. *Am J Respir Cell Mol Biol*. 1997;17(1):3-9.

45. Yamaguchi Y, Akizuki E, Ichiguchi O, et al. Neutrophil elastase inhibitor reduces neutrophil chemoattractant production after ischemia-reperfusion in rat liver. *Gastroenterology*. 1997;112(2):551-560.

46. Adcock IM. Transcription factors as activators of gene transcription: AP-1 and NF-kappa B. *Monaldi Arch Chest Dis* = Archivio Monaldi per le malattie del torace. 1997;52(2):178-186.

47. Hagiwara S, Iwasaka H, Hidaka S, Hasegawa A, Noguchi T. Neutrophil elastase inhibitor (sivelestat) reduces the levels of inflammatory mediators by inhibiting NF-kB. *Inflamm Res*. 2009;58(4):198-203.

48. Hayakawa M, Katabami K, Wada T, et al. Sivelestat (selective neutrophil elastase inhibitor) improves the mortality rate of sepsis associated with both acute respiratory distress syndrome and disseminated intravascular coagulation patients. *Shock*. 2010;33(1):14-18.

49. Miyoshi S, Ito R, Katayama H, et al. Combination therapy with sivelestat and recombinant human soluble thrombomodulin for ARDS and DIC patients. *Drug Des Devel Ther*. 2014;8:1211-1219.

50. Mohamed MMA, El-Shimy IA, Hadi MA. Neutrophil Elastase Inhibitors: a potential prophylactic treatment option for SARS-CoV-2-induced respiratory complications? *Crit Care*. 2020;24(1):311.