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New putative insights into neprilysin (NEP)-dependent pharmacotherapeutic role of roflumilast in treating COVID-19

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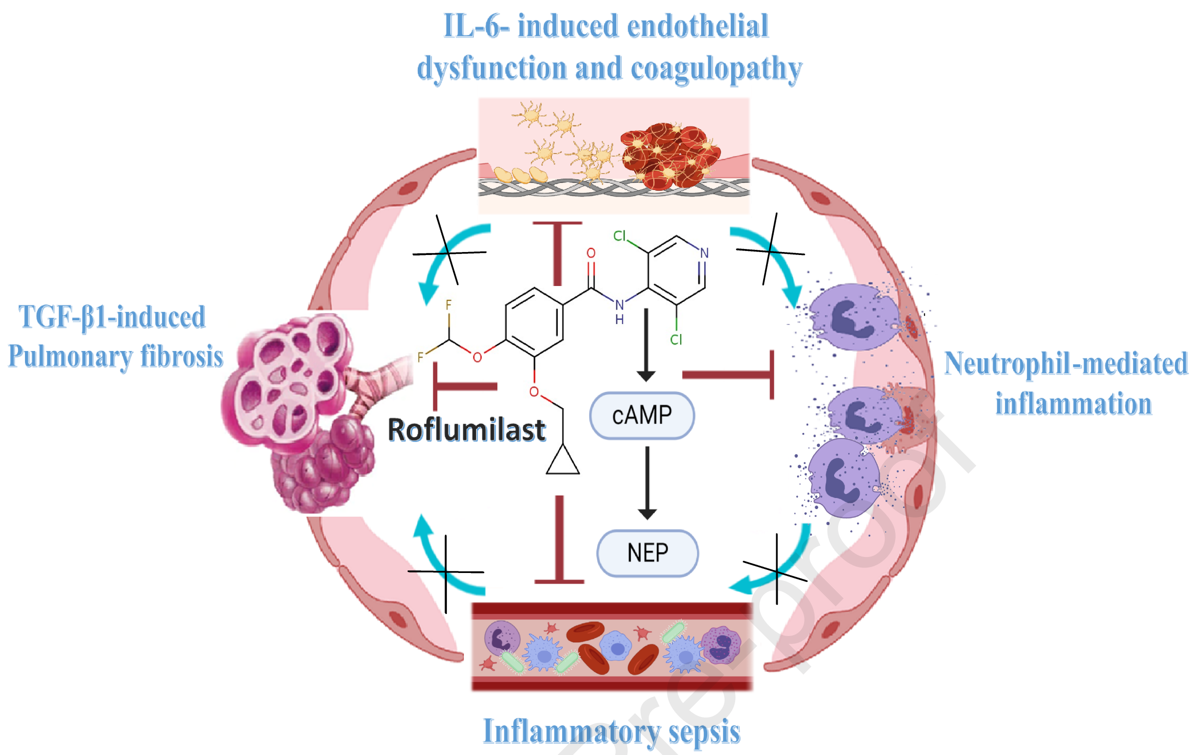
IL-6- induced endothelial dysfunction and coagulopathy
TGF-β1-induced Pulmonary fibrosis
Neutrophil-mediated inflammation
Roflumilast
cAMP
NEP
Inflammatory sepsis

Review article

New putative insights into neprilysin (NEP)-dependent pharmacotherapeutic role of roflumilast in treating COVID-19

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Abstract

Nowadays, coronavirus disease 2019 (COVID-19) represents the most serious inflammatory respiratory disease worldwide. Despite many proposed therapies, no effective medication has yet been approved. Neutrophils appear to be the key mediator for COVID-19-associated inflammatory immunopathologic, thromboembolic and fibrotic complications. Thus, for any therapeutic agent to be effective, it should greatly block the neutrophilic component of COVID-19. One of the effective therapeutic approaches investigated to reduce neutrophil-associated inflammatory lung diseases with few adverse effects was roflumilast. Being a highly selective phosphodiesterase-4 inhibitors (PDE4i), roflumilast acts by enhancing the level of cyclic adenosine monophosphate (cAMP), that probably potentiates its anti-inflammatory action via increasing neprilysin (NEP) activity. Because activating NEP was previously reported to mitigate several airway inflammatory ailments; this review thoroughly discusses the proposed NEP-based therapeutic properties of roflumilast, which may be of great importance in curing COVID-19. However, further clinical studies are required to confirm this strategy and to evaluate its in vivo preventive and therapeutic efficacy against COVID-19.

Keywords

COVID-19; Roflumilast; cAMP; Neprilysin; IL-6-induced endothelial dysfunction; Neutrophil-mediated inflammation; TGF-β1-induced pulmonary fibrosis

1. Introduction

COVID-19 is a global infectious disease that results in a huge number of deaths. For restricting its spread, there is an urgent need to evok the most effective therapy (Heng Li et al., 2020). Recently, a study hypothesizes that using anti-inflammatory PDE4i for modulating COVID-19 may be beneficial (Bridgewood et al., 2020). Among PDE4i, roflumilast exhibits the highest efficacy for targeting and blunting airway inflammation via enhancing the level of cAMP (Rabe, 2011), which in turn may prolong its anti-inflammatory effect by activating NEP (Graf et al., 1995). As NEP is lately supposed to be a new potential target for COVID-19 therapy (El Tabaa and El Tabaa, 2020), roflumilast-induced increase in NEP activity may have a prominent significance. Thus, we aim to review the proposed NEP-dependent pharmacological mechanisms by which roflumilast can block the inflammatory, coagulopathy and fibrotic cascades associated with COVID-19.

2. COVID-19 challenges

COVID-19 is a contagious fatal respiratory disease caused by a novel virus called severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). It was first recognized at the end of 2019 in Wuhan, China until being now an ongoing pandemic (Huang et al., 2020). As of 30 June 2020, more than 10.3 million cases have been reported across 188 countries and territories, resulting in more than 507,000 deaths and more than 5.28 million people have recovered (CSSE, 2020).

2.1 Clinical manifestations of COVID-19

Being one of severe airway diseases, COVID-19 patients usually show typical symptomatic respiratory presentations, such as cough, tiredness, muscle aches, headache, sore throat with sometimes fever and chills (Singhal, 2020). In such cohort, some patients may suffer from other worsened symptoms, such as profound acute shortness of breath combined with persistent chest pain, increasing the emergency need for oxygen therapy and mechanical ventilation (Yang et al., 2020). On the contrary, there are asymptomatic carrier states, who experience no symptoms or even only very mild symptoms; increasing thereby the risk of disease transmission (Lai et al., 2020).

Case reports declare that some people may display other unusual non-respiratory manifestations such as diarrhea which is recognized to be an initial sign of COVID-19 infection, in addition to taste or olfactory disorders which are especially identified in young people infected with SARS-CoV-2 (Luërs et al., 2020; Song et al., 2020).

Early clinical studies report that critically ill COVID-19 patients may associate with cardiovascular insults including myocardial injury, myocarditis, cardiac arrhythmias and heart failure with increased risk for thromboembolism as pulmonary embolus because of COVID-19-induced hypercoagulable state (Driggin et al., 2020).

Other cases with COVID-19 may also exhibit some neurological symptoms including dizziness, ataxia, altered mental state or even seizures (Mao et al., 2020). As well, some common COVID-19-related complications have been detected involving elevated liver enzymes, acute kidney injury (AKI) as well as an increased risk of developing fatal bacterial infections (Cox et al., 2020; Yang et al., 2020). Lately, ocular abnormalities such as conjunctival hyperemia, chemosis, and increased secretions are additionally reported in COVID-19 infected patients (Wu et al., 2020).

2.2 High-risk groups of COVID-19

As documented, COVID-19 can infect different groups of people, where most of them will recover without hospitalization, but others will develop sever complications. People at higher risk from COVID-19 include older people, usually over 60 to 70 years old and those who have weakened immune response either due to administering chemotherapy, radiation or medication for an autoimmune disease, undergoing an organ or stem cell transplant, losing a spleen or having a non-functioning one. Moreover, adults (over 18 years old) with underlying chronic medical conditions such as high blood pressure, diabetes, chronic heart, lung and kidney diseases are more vulnerable to succumb to COVID-19 infection (Vishnevetsky and Levy, 2020). Similarly, pregnant women appear to be more susceptible to COVID-19 with the potential of developing maternal and fetal complications (H. Liu et al., 2020). As well, there is also an increased risk for overweight people and heavy cigarettes smokers (Tamara and Tahapary, 2020; van Zyl-Smit et al., 2020).

On the other hand, all children, even those with underlying medical problems, did not show a high risk of severe illness from COVID-19 (Lyu et al., 2020).

3. Pathophysiology of COVID-19

Since the prevalence of COVID-19 has nowadays become a major global burden around the world, there has been a necessity to perform the precious pathophysiological researches that will aim at recognizing the involved biological markers and the clear mechanisms through which the disease pathogenicity induced by SARS-CoV-2 can be explained.

Obviously, the coronavirus genome cannot be replicated outside the cytoplasmic membranes, so it continuously seeks to penetrate living cells for ensuring its survival. For viral replication, polyproteins should be firstly hydrolyzed into functional proteins by a variety of proteolytic enzymes, which are more commonly known to RNA viruses such as RNA-dependent RNA polymerase (RdRp), 3 chymotrypsin like protease (3CL protease), papain like protease and helicase (Ziebuhr, 2005).

At present, several studies showed that penetrating pneumocytes is considered as the main pathway for SARS-CoV-2 replication within the human body. That finding is ensured from the evidence of utilizing angiotensin-converting enzyme 2 (ACE-2) enzyme as receptors for viral entry, **(Fig. 1)** (H. Zhang et al., 2020). ACE-2 was found to be highly expressed in alveolar and bronchial membranes, in type II pneumocytes and possibly on vascular endothelial cells (EC) within lungs (Jia, 2016); explaining why the common signs and symptoms of respiratory infection will develop in coinciding with COVID-19 disease.

Simultaneously, ACE-2 protein was also detected to be distributed in various human organs other than lungs involving oral and nasal mucosa, gastrointestinal tract (GIT), skin, heart, liver, kidney, and brain (Hamming et al., 2004); elucidating the reason for developing other extra-pulmonary manifestations associated with COVID-19 infection.

Binding of SARS-CoV-2 with ACE-2 may downregulate ACE-2 and subsequently, inhibit the ACE-2-regulated generation of angiotensin (1–7) peptide which can, via Mas receptor, perform several beneficial activities as vasodilator, anti-inflammatory, anti-hypertrophy, anti-proliferative, anti-fibrosis and antioxidant (Kuba et al., 2005).

Concerning the pulmonary RAS, cutting off the ACE-2 / angiotensin (1–7) / Mas receptor axis will activate the vasopressor ACE / angiotensin (Ang) II / angiotensin II type 1 receptor (AT1) axis on the other side. The axis which may drive the airway inflammatory cascades, because of significant increase in Ang II level. Ang II, through activating angiotensin II type 1 receptor, could promote the release of multiple inflammatory cytokines especially TNF-α, IL-6, GM-CSF and MCP-1 (Sprague and Khalil, 2009).

3.1 Cytokine storm in COVID-19

Cytokine storm is a fierce interplay of cytokines that can occur in numerous infectious and non-infectious diseases (Teijaro, 2017). It is considered as a potentially fatal immune reaction that consists of a positive feedback loop between cytokines and immune cells. When the immune system is fighting pathogens, cytokines signal immune cells, such as T cells and macrophages can travel to the site of infection, where they will be activated and stimulated to produce more cytokines. This positive feedback loop reaction becomes uncontrolled and then, too many immune cells are activated in a single place. Consequently, cytokine storm will have the potential to significantly damage body tissues and organs (Tisoncik et al., 2012).

In the lungs, for example, increasing the release of cytokines such as interleukin-6 (IL-6) will trigger the fluids and immune cells to be accumulated, eventually block off the airways, and potentially lead to death (Rincon and Irvin, 2012) . This is obviously detected in seriously ill COVID-19 patients who showed high levels of IL-6 (Dal Moro and Livi, 2020).

Because of the positive correlation between high IL-6 level and COVID-19 severity, IL-6 is specifically suggested to be the master marker used for monitoring disease progression (T. Liu et al., 2020). There is a growing evidence that IL-6 can play a crucial part in the uncontrolled intestinal inflammatory process, proving its role in the pathogenesis of COVID-19-asociated diarrhea. However, another causing factor may be attributed to the direct viral invasion of gut epithelial cells via ACE-2 (Mudter and Neurath, 2007).

As previously reported, IL-6 could prohibit the olfactory signal pathway; proposing that anosmia detected in COVID-19 patients may be due to IL-6-mediated inflammation of the nasal mucosa (Henkin et al., 2013; Luërs et al., 2020). Besides, other additional elements supporting that SARS-CoV-2 may have a neuro-invasive propensity to invade the central olfactory pathway causing olfactory dysfunction (Marinosci et al., 2020). Jointly, IL-6 was also found to be extremely involved in promoting the ocular inflammation; matching with conjunctivitis that is recently reported to be linked with COVID-19 infection (Ghasemi, 2018).

3.2 IL-6-induced endothelial dysfunction and coagulopathy in COVID-19

In addition to the direct role of SARS-CoV-2/ACE-2 interaction in inducing the endothelial dysfunction (Y. Zhang et al., 2020), IL-6 was also reported to interrupt the normal function of endothelial cells (ECs) through inactivating the endothelial nitric oxide synthase (eNOS) which in turn could decrease NO production with subsequent induction of an oxidative stress state leading to impairment in endothelial responses (Hung et al., 2010).

As a consequence, disrupting the endothelial cell function either by SARS-CoV-2 itself or IL-6 could activate the platelets and stimulate their adhesion and aggregation; resulting in a pulmonary specific vasculopathy termed pulmonary intravascular coagulopathy (PIC) (Aird, 2003; Levi and van der Poll, 2017; McGonagle et al., 2020).

Most anatomical studies of COVID-19 victims demonstrate the formation of blood thrombus (fibrin clot) in their pulmonary vessels, in addition to deep vein thrombosis that increases the risk for developing pulmonary embolism (Cui et al., 2020; Klok et al., 2020). These clots result in a compensatory increase of plasminogen (fibrinolysin) but, with disease progression, it fails to break down these fibrin deposits reflected in elevated D-dimer (DD) levels, which is reported to be associated with the severity of COVID-19 infection and may be also correlated with activation of the pro-inflammatory cytokine cascade (Belen-Apak and Sarıalioğlu, 2020; Leonard-Lorant et al., 2020).

Emerging data suggest that COVID-19-associated endothelial dysfunction could induce several structural and functional changes resulting in leukocyte trafficking, which in turn, may shift the vascular equilibrium towards triggering more inflammation (Aird, 2003). Although leukocyte trafficking was known to play an essential part in the protective responses against any infection or injury, it may also lead to extensive tissue damage as shown in numerous inflammatory disorders (Chen et al., 2018). One of the most abundant leukocytes being assured in COVID-19 are neutrophils that represent the first line of defense in the innate immune system.

3.3 Neutrophil-mediated inflammation in COVID-19

With the continual reduction detected in lymphocytes count of COVID-19 patients, they become more prone for secondary infections with the risk of high mortality rate. This occurs due to loss of all lymphocyte effector cells that possess the essential antiviral activity, including CD8+ or cytotoxic lymphocytes and natural killer cells, as well as B cells, which able to form the specific antibodies targeted for inactivating the virus (Dallan et al., 2020; Remy et al., 2020).

Therefore, developing severe lymphopenia will effectively inhibit the stimulation of adaptive cell-mediated immune response and consequently, facilitate the inflammation-mediated neutrophil response which could be started with their chemotaxis and recruitment, followed by degranulation (Didangelos, 2020; Hyun and Hong, 2017). Neutrophils possess an arsenal of proteases such as (elastase, proteinase-3 and cathepsin G), inflammatory mediators such as (TNF-α and IL-6), and toxic oxidants that do not kill phagocytosed pathogens only, but also can damage the host tissue (Gernez et al., 2010).

3.4 Inflammatory sepsis in COVID-19

In response to high neutrophilia with progressive lymphopenia established in COVID-19, viral sepsis may be promoted as a result of systemic uncontrolled inflammation induced by neutrophils with further worsening of tissue injury (Hui Li et al., 2020), that is consistent with the final diagnosis emphasizing the existence of a septic shock among COVID-19 patients with profound lymphopenia (Dallan et al., 2020).

Sepsis is a syndrome that has attracted the attention worldwide because of its high mortality rate of about 50–80%. It is widely recognized as a systemic inflammatory response syndrome, that had been defined as a complex disorder arising from the dysregulation of an inflammatory response of the entire organism to an infection or to circulating bacterial products, rather than infection (Bone et al., 1992). However, sepsis has been now redefined as a life-threatening organ dysfunction due to a dysregulated response of the host to infection (Singer et al., 2016).

Sepsis itself may share in the subsequent release of inflammatory factors (IL-6 and TNF-α) that could eventually aggravate the existing inflammation (Molano Franco et al., 2019) and thus, could lead to multiple organ dysfunction, shock, and even death, which are not caused directly by the invading pathogens; but as a result of inflammation (Crowther, 2001; Mantzarlis et al., 2017).

During sepsis, there is an extensive crosslink between increased inflammation, endothelial dysfunction and hyper-coagulopathy, in which the microvascular dysfunction was documented to be one of important sepsis hallmarks (Schouten et al., 2008).

3.5 TGF-β1-induced pulmonary fibrosis in COVID-19

Given the reported evidence of induced endothelial dysfunction, pulmonary fibrosis may be also prompted as a substantial problem during COVID-19 infection, to the extent that pulmonary post-mortem findings in fatal cases of COVID-19 revealed the presence of extensive fibrotic features as myofibroblastic proliferation or organizing pneumonia (George et al., 2020). The vascular endothelial dysfunction could stimulate the fibrotic consequences via secreting a peptide, namely endothelin-1 (ET-1) (Elshazly et al., 2013), which could induce the release of transforming growth factor- β1 (TGF-β1), a fibrogenic cytokine mainly implicated in driving the pulmonary fibrosis development (Wermuth et al., 2016).

3.6 ET-1-reduced cAMP in COVID-19

Surprisingly, ET-1 is also suggested to exaggerate the inflammation via inhibiting adenylyl cyclase (AC) activity and thereby, cAMP accumulation (Insel et al., 2012).Within the immune system, cAMP is synthesized from ATP by the action of AC to regulate the anti-inflammatory effects (Gentile et al., 1988). As reported, cAMP could decrease the production of pro-inflammatory mediators as well as enhance the production of anti-inflammatory factors in various immune cells (Raker et al., 2016). Meanwhile, cAMP was concluded to promote ATP production that is described to potentially improve the efficiency of innate and adaptive immune systems for fighting off COVID-19 (De Rasmo et al., 2016; Taghizadeh-Hesary and Akbari, 2020).

Consistent with these findings, it was reported that COVID-19 may be more fatal in the elderly-population than in children, as with increasing the age, there is a gradual decline in the cellular ATP and subsequent ATP-induced cAMP accumulation (Srivastava, 2017). Furthermore, tobacco smokers, who suffer from a decreased content of ATP in immune cells, are also found to be more susceptible for COVID-19 infection (Malińska et al., 2019).

Regardless of age, males are generally more prone to die by COVID-19 than females (Jin et al., 2020). The finding which can be attributed to sex hormone differences, since estrogen was recorded to potentially induce ATP production during the inflammation than androgens (Kassi and Moutsatsou, 2010).

Additionally, the same strategy could be particularly relevant for patients with serious medical conditions, who showed an immune dysregulation as a result of ATP-depletion (Zhou et al., 2020).

4. COVID-19 therapies

With extremely rapid increase in the number of SARS-CoV-2- infected cases globally, there is unfortunately sufficient time for discovering a newly therapeutic agent. Taken together, directing most efforts towards vaccine production may be of no avail at least nowadays, since millions of people everywhere have been already infected with COVID-19, and they are in urgent need for rapid treatment in order to prevent the disease progression. In addition, developing anti-viral drugs needs a long way to go. Therefore, the best choice may be repurposing the currently available drugs which may greatly save time and money as well as secure many people from death.

World Health Organization (WHO) reported that COVID-19 now becomes much more than a health crisis. Till present, curing COVID-19 remains elusive, in spite of the great efforts directed by the researchers towards understanding and identifying the disease mechanisms. There is no doubt that COVID-19 can trigger airway inflammatory reactions, in which neutrophils play the major role in increasing the severity by inducing COVID-19-associated coagulopathy (Zuo et al., 2020). In that context, several therapeutic strategies have been proposed to control COVID-19 (Cascella et al., 2020).

4.1 Current therapies

The most common one involves the use of hydroxychloroquine (HCQ) as the first-line therapy because of its anti-inflammatory and immunomodulatory effects (Hu et al., 2017). Based on the international guidelines, HCQ is reported to be utilized either alone or in combination with other drugs including, systemic corticosteroids, tocilizumab (TCZ), macrolide azithromycin, antiviral lopinavir/ritonavir and anticoagulant enoxaparin (Mehra et al., 2020; Rosenberg et al., 2020). However, the use of HCQ is lately recorded to have many restrictions due to increased risk of serious cardiac arrhythmias (Nguyen et al., 2020). Additionally, both HCQ and chloroquine (CQ) are no longer authorized by FDA to treat COVID-19 (FDA.,2020).

Moreover, current COVID-19 treatment protocol also recommends the use of oral anti-inflammatory steroids such as dexamethasone or inhaled corticosteroid such as ciclesonide. Ciclesonide was reported to exhibit both antiviral and anti-inflammatory actions with less systemic immunosuppressive effects (Matsuyama et al., 2020). However, further studies are needed to confirm its potential effect against COVID-19 (Iwabuchi et al., 2020).

Controversially, using steroids may paradoxically exaggerate the COVID-19-associated neutrophilia (Fukakusa et al., 2005). In addition, steroids should be taken with caution in vulnerable patients with pre-existing hypertension, diabetes, or cardiovascular diseases, which, at the same time, represent the highest risk group of COVID-19 (Varga et al., 2020). That pushed clinicians to search for additional or alternative anti-inflammatory treatments that can efficiently control the neutrophilic component of COVID-19 apart from steroid related complications. TCZ, a humanized monoclonal antibody acting by blocking IL-6 receptor, has been suggested for COVID-19 patients to suppress the inflammatory storm and minimize the mortality (Fu et al., 2020). However, some studies showed that TCZ may effectively reduce both fever and inflammatory markers, but with no satisfactory clinical outcomes inferred for the critically ill COVID-19 patients (Campochiaro et al., 2020; Dastan et al., 2020). As documented, this medication may also raise both blood pressure and lipid levels, which are considered the main risk factors exaggerating the severity in COVID-19 patients of cardiovascular (CV) diseases (Rao et al., 2015). Furthermore, anti-interleukin therapy is expected to worsen the post-COVID-19 pulmonary fibrosis (George et al., 2020; Silva et al., 2020).

As regards to azithromycin, pieces of clinical evidence revealed that it could exert a great role against both SARS and Middle East Respiratory Syndrome (MERS), that prompted scientists to strongly suggest it as a potential treatment for COVID-19. Azithromycin was detected to possess anti-inflammatory and immunomodulating actions in addition to antiviral properties because of its ability to minimize the production of pro-inflammatory cytokines particularly IL-6 and TNF-α, noxious oxidative radicals as well as to improve T-helper cell functions. However, the preliminary studies have demonstrated that using azithromycin should be in caution due to its potential arrhythmogenic threat, especially in high risk COVID-19 patients (Pani et al., 2020).

Moreover, provision should be also taken to mitigate the cardiac risk, especially after adding lopinavir/ritonavir into the current treatment protocol for COVID-19 (Gérard et al., 2020). Lopinavir acts as anti-HIV protease inhibitor via inhibiting the action of 3CLpro, thus disrupting the viral replication and release from host cells. Recent in vitro study indicates that lopinavir can also exhibit antiviral activity against SARS-CoV-2, with which ritonavir can be added as a booster. However, there is a contradictory survey having concluded that the use of lopinavir/ritonavir shows no significant reduction in the mortality rate within the severely ill COVID-19 patients (Owa and Owa, 2020).

A prodrug of adenosine analogue, namely remdesivir has also shown antiviral activity against COVID-19 in human airway epithelial cells and in a non-human primate model. Because of its efficacy in inhibiting viral RNA-dependent RNA polymerase, remdesivir had previously prescribed as a broad-spectrum antiviral agent for several RNA viruses such as respiratory syncytial virus, Nipah virus, Ebola virus (EBOV), MERS-CoV, and SARS-CoV-1 (Singh et al., 2020).

A novel originally developed broad-spectrum antiviral drug, favipiravir, has been also experimentally tested against COVID-19. Favipiravir is a pyrazine carboxamide derivative that can selectively block influenza viral replication via inhibiting the viral RNA-dependent RNA polymerase (Cai et al., 2020).

Additionally, nafamostat, an oral serine protease inhibitor, was reported to significantly inhibit SARS-CoV-2 infection in lung-derived human cell line Calu-3 (Hoffmann et al., 2020). Regarding the efficacy and safety of nafamostat, a prospective clinical trial (NCT04352400) is being conducted to evaluate its possible role against COVID-19 (Azimi, 2020).

Another repurposed drug suggested for treating COVID-19 because of its potential antiviral activity was famotidine. Using famotidine, a histamine-2 (H2RA) receptor antagonist among the hospitalized COVID-19 patients was documented to reduce the mortality rate. Famotidine may interfere with SARS-CoV-2 maturation by inhibiting the activity of 3CLpro. However, its therapeutic role against COVID-19 is still at nascent stage and randomized controlled trials are urgently needed (Aguila and Cua, 2020).

4.2 Potential COVID-19 therapies

Considering ACE-2 to be the only viral receptors, a new study has proposed that lactoferrin, an orally nutritional supplement, may be potentially useful against COVID-19. In addition to its unique immunomodulatory and anti-inflammatory effects, lactoferrin has been described to possibly occupy angiotensin-converting enzyme ACE-2 receptors preventing SARS-CoV-2 from attaching to the host cells (Kell et al., 2020), however it is not proved till now.

Most of the repurposed drugs used for treating COVID-19 are directed mainly towards blocking the induced cytokine storm, however this COVID-19-related sepsis argues now for investigating a different therapeutic approach (Remy et al., 2020).

Since the morbidity/mortality rate in septic patients was reported to be correlated with the plasma level of ET-1, reducing its level may minimize all unwanted reactions mediated by endothelin ET-1 receptors. The observation that may explain why anti-inflammatory drugs like anti-TNF-α and IL-1-based therapies have failed in treating sepsis, opposite to clinical trials that indicated the application of endothelin ET-1 receptor blockers as an effective strategy (Kowalczyk et al., 2015). In addition, decreasing ET-1 level may interrupt the fibrotic pathway regulated by TGF-β1, thus inhibiting the induction of pulmonary fibrosis.

Because ET-1 was previously reported to be one of the substrates that could be potentially degraded by endogenous NEP (neutral endopeptidase) (Abassi et al., 1992), that pushed us to predict that enhancing NEP activity may become a prerequisite to defeat COVID-19 ghost (El Tabaa and El Tabaa, 2020).

NEP is a type II integral transmembrane metallopeptidase, which was clearly detected in various tissues like lung, kidney, brain, intestine, and vascular endothelium (Li et al., 1995) as well as in many inflammatory cells including neutrophils (Connelly et al., 1985). In the airways, NEP has been found to be expressed in the epithelium (Sont et al., 1997), smooth muscle cells (Di Maria et al., 1998), and fibroblasts (Kletsas et al., 1998).

NEP was also found to degrade the endogenous vasoactive peptides including atrial natriuretic peptide (ANP). Thus, inhibiting NEP can prolong and potentiate their natriuretic actions. That action pushed clinicians to use NEP inhibitors (e.g. Sacubitril) in a combination with ACE inhibitors (e.g. valsartan) for lowering blood pressure and treating heart failure (Bratsos, 2019).

Furthermore, a high cleaving affinity of NEP towards some potent inflammatory such as bradykinins (BKs) and N-formyl-L-methionyl- L-leucyl-L-phenylalanine (fMLP) emphasized its potential role in alleviating the airway inflammatory processes (Connelly et al., 1985; Shimamoto et al., 1994).

Several studies ensured that destroying or down-regulating NEP may lead to further pathophysiological changes. This involves an increase in vascular permeability, recruitment, and activation of inflammatory cells, particularly neutrophils. Neutrophil chemotaxis will lead to the release of neutrophil elastase enzymes (e.g., cathepsin G), which may exert further destructive effects on airway tissues, leading to worsening and progression of the disease (Borson, 1991).

Therefore, reducing NEP activity either by cigarette smoking (Dusser et al., 1989), hypoxia (Carpenter and Stenmark, 2001) or respiratory pathogens like parainfluenza virus type 1, rat corona-virus, and Mycoplasma pulmonis (Borson et al., 1989; Jacoby et al., 1988), will be a clear explanation for their associated inflammatory cascades. Considering multiple activities of NEP in regulating local inflammatory neuropeptides within alveolar microenvironment and nearby vascular cells (Wick et al., 2011), it may exhibit a good target for counteracting the airway inflammation, coagulopathy and pulmonary fibrosis associated with COVID-19 infection.

Referring to the studies searching for agents that may up-regulate NEP gene expression; enhancing its activity and promoting its action (Borson, 1991), a variety of selective enhancers are pre-clinically developed involving drugs (glucocorticoids) (Borson and Gruenert, 1991), hormones (androgens (Yao et al., 2008) and estrogen (Xiao et al., 2009)) or natural products (apigenin, luteolin, and curcumin, epigallocatechin and resveratrol) (Ayoub and Melzig, 2008; Chang et al., 2015; El-Sayed and Bayan, 2015).

Along with this line, Rolipram, an investigative PDE4i, has also been examined, since the increase in intracellular cAMP levels correlate directly with enhanced NEP activity, which in turn may prolong and potentiate the cAMP-mediated short-term anti-inflammatory mechanism (Ayoub and Melzig, 2008; Graf et al., 1995). This outcome implies that another selective PDE4i, roflumilast, could exert efficient anti-inflammatory effect via elevating cAMP level as well as NEP activity. Accordingly, we predict that roflumilast may be one of the most useful drugs that is expected to play a great role in treating COVID-19. However, until this moment, no study has indicated the potential fundamental pathways contributing to relying roflumilast on NEP activity.

5. Roflumilast overview

Roflumilast is recorded to be a highly selective long-acting inhibitor of PDE4 isoenzyme, to which its use will be surely accompanied with an increase in the level of intracellular cAMP (Rabe, 2011).

5.1 Phosphodiesterase enzymes (PDEs)

Phosphodiesterase enzymes (PDEs) are a large superfamily of enzymes that catalyze the hydrolysis of second messengers such as cAMP and cyclic guanosine mono-phosphate (cGMP) into their inactive 5′ monophosphate; thus regulating their intracellular level as well as the amplitude and duration of their signaling (Hertz et al., 2009).

Based on amino acid sequences, tissue distribution and pharmacological properties, PDEs could be classified into 11 sub-families, namely PDE1-PDE11. Similarly, PDEs can be also grouped into three categories according to their substrate specificities including, cAMP-selective hydrolases (PDE4, 7 and 8), cGMP-selective hydrolases (PDE5, 6, and 9) and hydrolases for both cAMP and cGMP (PDE1, 2, 3, 10, and 11) (Azevedo et al., 2014).

Regarding PDE4, it was accounted to represent the predominant isoenzyme responsible for regulating cAMP levels in many cell types within the lung including airway epithelial cells, airway smooth muscle cells and pulmonary vascular endothelium. PDE4 was also noticed to be widely distributed in various inflammatory cells, like neutrophils, T lymphocytes, eosinophils, monocytes and basophils (Halpin, 2008; van Schalkwyk et al., 2005).

Notably, cAMP has a direct significant role in different inflammatory pathways via inhibiting ROS generation and pro-inflammatory cytokine production, mainly TNF-α and IL-6 (Isoni et al., 2009; Shames et al., 2001). cAMP could also promote the production of anti-inflammatory mediators such as IL-10 which was identified as a “cytokine synthesis inhibitory factor”, and acted as a principal regulator in the JAK-STAT signaling pathway (Redford et al., 2011). Therefore, elevating cAMP level within the pulmonary tissue, vascular and inflammatory cells can provide an efficient anti-inflammatory action (Li et al., 2018).

On the other hand, it was found that the capacity of PDEs for cAMP hydrolysis is greater than the maximum rate of its synthesis. Therefore, minute reduction in PDEs activity can result in a high elevation in cAMP level with significant changes in the activity of its dependent protein kinase (Halpin, 2008). That notice pushed scientists since 1970 to investigate the potential therapeutic importance of inhibiting PDE4 activity (Weiss and Hait, 1977).

5.2. Selective and non-selective PDE4i

Because of the involvement of cAMP signaling in the pathophysiology of many inflammatory diseases, it has been proved that targeting PDE4 will resemble an effective therapeutic strategy for different inflammatory conditions, such as chronic obstructive pulmonary disease (COPD), asthma, atopic dermatitis (AD), inflammatory bowel diseases (IBD), rheumatic arthritis (RA), lupus and neuroinflammation (Li et al., 2018).

Early, non-selective PDE inhibitors were discovered including theophylline and doxofylline, but, because of their associated significant adverse effects, their use had been limited.

Given that PDE4 is the only cellular pathway available for cAMP degradation (Fertig, Bracy A., 2018), therapeutic studies have been directed to develop the most selective PDE4 inhibitors, among which, apremilast and roflumilast are currently available (Boswell-Smith et al., 2006; Kumar et al., 2013).

6. Pharmacotherapeutic effects of roflumilast

Since 2011, roflumilast has been approved by FDA as an anti-inflammatory drug specifically designed for many respiratory disorders mainly COPD and asthma. By time, roflumilast has been reported to exert different pharmacological activities, **Figure 2 and Table 1** (Li et al., 2018).

6.1 Roflumilast and lung inflammation

Clinical trials have shown that that oral administration of roflumilast could suppress airway inflammation and improve lung function of COPD patients. In addition, it is documented to be effective in reducing the frequency of disease exacerbations when given as add-on to inhaled therapy in patients with moderate or severe COPD (Shen et al., 2018). As regards asthmatic patients, roflumilast could also significantly increase the Forced expiratory volume in 1 s (FEV_1) and improved airway inflammation (Bateman et al., 2006).

The anti-inflammatory mechanisms of roflumilast can be contributed to its PDE4 inhibiting activity, leading to an increase in cAMP concentration and signaling within the epithelial airway and inflammatory cells. The action which in turn will enable roflumilast to suppress the expression of pro-inflammatory cytokines such as IL-6 and TNF-α (Feng et al., 2017). Moreover, another study of cigarette smoke-induced pulmonary inflammation in guinea pigs showed that roflumilast could effectively reduce the numbers of neutrophils, lymphocytes and eosinophils in bronchoalveolar lavage fluid (Fitzgerald et al., 2006).

For COPD patients, roflumilast was represented to exert a significant role in reducing eosinophil cell counts within their bronchial biopsy samples and sputum (Rabe et al., 2018), in addition to its direct suppressing effect on neutrophils function and their ROS production. As a result of elevating cAMP level, roflumilast could inhibit neutrophil chemotaxis and degranulation. cAMP could directly activate protein of Epac1, which in turn could suppress neutrophil migration as well as oxidative burst. Furthermore, cAMP could also activate protein kinase A (PKA) in neutrophils, leading to a decline in their phagocytic activity (Dunne et al., 2019).

Some in vivo and in vitro studies revealed that roflumilast can potently reduce the endothelial permeability and suppress the leukocyte–endothelial cell interactions through altering the expression of adhesion molecules and attenuating the up-regulation of polymorphonuclear leukocytes (PMNL) surface CD11b, that may be stimulated either by fMLP or platelet-activating factor (PAF). That action could inhibit neutrophil adhesion to endothelial cells (Sanz et al., 2007). Additionally, results from in vitro studies of human neutrophils showed that roflumilast could prevent the release of neutrophil elastase, matrix metalloproteinase and myeloperoxidase, inhibiting neutrophil function (Jones et al., 2005)

A synergistic effect of roflumilast with other anti-inflammatory agents such as corticosteroids or long-acting β2-agonists have been demonstrated (Kawamatawong, 2017). It was concluded that roflumilast-N-oxide (RNO), the active metabolite of roflumilast, could enhance the anti-inflammatory effect of dexamethasone in airway smooth muscle cells in vitro (Patel et al., 2017). At the same time, roflumilast was reported to reverse the corticosteroid-associated insensitivity towards neutrophils in COPD patients (Milara et al., 2015b). As well, other study revealed the great value of roflumilast in restoring the glucocorticoid sensitivity in glucocorticoid-resistant patients through blocking the downregulation of glucocorticoid receptor (GRα) alpha, which was known to be responsible for glucocorticoid resistance (Reddy et al., 2020).

6.2 Roflumilast and hypercoagulable states

Neutrophils and platelets have been identified as crucial factors for thrombus initiation and progression. Both animal models and human diseases increased the evidence that neutrophils extracellular traps (NETs) possess a significant role in the pathogenesis of thrombosis. NETs were detected to be released from the activated neutrophils in a process called NETosis, which can be mediated by recruitment of both platelets and PMNL into the endothelial wall. Then, NETs could stimulate platelet adhesion, activation and aggregation with subsequent activation of coagulation cascades to trigger thrombosis (Fuchs et al., 2010; Kimball et al., 2016).

Accordingly, inhibiting the prothrombotic function of neutrophils and interfering with NETs formation by roflumilast, could reduce the risk of thrombosis in COPD as well as in other inflammatory diseases. Moreover, RNO (an active metabolite of roflumilast) was recorded to affect NETs via inhibiting Src family kinases phosphoinositide 3-kinase (SFK–PI3K) pathway in PMNs. In addition, RNO could block the key biochemical mechanisms regulating PMN–platelet adhesion *(Totani et al., 2016).*

6.3 Roflumilast and inflammatory sepsis

Janus kinase (JAK)/Signal transducer and activator of transcription-3 (STAT-3) constitute a key cellular signal transduction pathway for mediating the expression of many inflammatory cytokines produced during sepsis (Cai et al., 2015). This pathway resembles a positive feed-back signal for exacerbating the inflammatory response, resulting in uncontrolled systemic inflammation (Chang et al., 2019). Moreover, during sepsis, there is also an inflammation-induced activation of coagulation as a result of the concomitant impairment of endothelial function, anticoagulant and fibrinolytic systems, indicating that systemic inflammation will be the main pathological reaction of sepsis and the major cause for associated multiple organ failure (Schouten et al., 2008). Therefore, reducing inflammation could be the key for treating sepsis.

Regarding the role of roflumilast in suppressing the mRNA expression of JAK/STAT-3 signaling pathway with subsequent inhibition of inflammatory cytokine release (e.g. IL-6 and TNF-α) in the lung tissue of septic mice model (Chang et al., 2019), there is a proof of its potential therapeutic benefits in septic organ dysfunction through the above-referred anti-inflammatory and anti-thrombotic activities (Hattori et al., 2017).

6.4. Roflumilast and lung fibrosis

Because of the potential effect of anti-inflammatory treatment to mitigate airway fibrotic remodeling, roflumilast might play anti-fibrotic role due to its well-known anti-inflammatory action (Hatzelmann et al., 2010).

Roflumilast was found to have the ability to prevent the progressive airway fibrosis, as a result of antagonizing fibroblast activity, which could be mediated by

TGF-β1, an essential regulator of immune responses related to fibrosis (Togo et al., 2009). Anti-fibrotic profile of roflumilast could be also explained by its ability to reduce the expression of upregulated NADPH oxidase 4 (NOX4) (Milara et al., 2015c), which was indicated to be critical for pulmonary fibrotic remodeling (Amara et al., 2010).

Within this regard, roflumilast could also normalize most of increased metabolic changes like alterations in oxidative equilibrium, increased collagen, and protein synthesis, resulting in decline in the fibrotic score. Simultaneously, reduced lung tissue pH has been proposed as a risk factor for lung fibrosis development, which was also reported to be corrected by roflumilast in bleomycin model of pulmonary fibrosis (Milara et al., 2015a).

7. Adverse effects and safety of roflumilast

Roflumilast can be safely administered as it is not associated with the parlous induction of adverse effects involving seizures and cardiac arrhythmias; in addition, its elimination is not significantly altered by several drug classes or even by food and tobacco smoking (Gupta and O'Mahony, 2008).

However, results from clinical trials demonstrated that the anti-inflammatory dose of roflumilast in human was reported to be associated with a set of minor side effects such as nausea, vomiting, diarrhea, weight loss and headache (Baye, 2012). These effects appeared to be dose-dependent and transient, which in turn did not need treatment discontinuation (van Schalkwyk et al., 2005). As such, the newly drug developing strategies are being directed to improve the therapeutic index of roflumilast.

Great efforts have been made to limit the gastrointestinal adverse reactions and to provide a better benefit (Li et al., 2018). Thus, for improving patient tolerability, a study in the allergen-challenged Brown Norway rats, has been performed to evaluate the efficacy of inhaled roflumilast given either intratracheally or by nasal inhalation. As concluded, the inhaled form showed a powerful effect on improving the lung function (Chapman et al., 2007), supporting the therapeutic importance of using inhaled PDE4i against inflammatory lung diseases, which may be then more efficacious with fewer adverse effects than its oral forms, however it is still under clinical trial (Rhee and Kim, 2020).

8. Roflumilast in aging, diabetic, and cardiovascular comorbidities

During physiological aging process, a low-grade chronic systemic inflammation, called inflammaging, develops and impairs the maintenance of immunological homeostasis, in which there are high levels of C-reactive protein (CRP), proinflammatory cytokines as IL-6, in addition to low level of anti-inflammatory cytokines as IL-10 (Franceschi et al., 2018). PDE4 enzymes play a major role against inflammaging by increasing cAMP which in turn stimulates AMP-activated protein kinase (AMPK), exerting an anti-inflammatory effect. Since PDE4 enzyme activity in elderly individuals is greater compared with the activity in younger subjects, using roflumilast can experience a relatively more increase in cAMP level and as a consequence, potentiate its anti-inflammatory action in old age people (Muo et al., 2019).

Given the essential role of PDE4 in glucose and fat metabolism, roflumilast, through PDE4 inhibition, could prevent the disease progression in diabetes mellitus (DM) type 2 patients via improving the glycemic index. Roflumilast could encourage the secretion of intestinal glucagon like peptide-1 (GLP-1), which is a main incretin with effective insulinotropic action on pancreatic beta cell (Wouters et al., 2012). In addition, it was documented that a deficiency in PDE4B could attenuate high-fat diet-induced adiposity and adipose tissue inflammation in mice (Vollert et al., 2012), referring to the role of roflumilast in reducing weight and improving insulin sensitivity in adults with prediabetes and/or obesity (Muo et al., 2019).

For cardiovascular safety, roflumilast showed a lower rate of major adverse cardiovascular events in treated COPD patients, supposing its potential cardiovascular benefits (Rogliani et al., 2016; White et al., 2013).

9. Roflumilast and COVID-19 infection

The rationale for selecting PDE4i for COVID-19 may be based on the previous findings demonstrating that inhibiting the activity of PDE4 will suppress a myriad of pro-inflammatory responses (Press and Banner, 2009). Inhibiting PDE4 will specifically prevent cAMP degradation, which in turn will decrease airway inflammation via preventing the activation and recruitment of inflammatory cells, specifically neutrophils as well as cytokines production (Barnette, 1999). That observation drives scientists to attractively target PDE4 for treating COVID-19.

In addition to its anti-inflammatory, anti-coagulant and anti-diabetic roles, roflumilast could be used safely in a combination with corticosteroids, recommended to be used effectively against COVID-19 infection, by improving their compromised anti-inflammatory properties and their resistance effect (Milara et al., 2015b; Wang et al., 2016).

At the same time, azithromycin, a macrolide antibiotic suggested for COVID-19 treatment, was documented to exhibit a lower affinity for cytochrome P-450A (CYP) 3A4 CYP 3A4. Thus, azithromycin would poorly interact with roflumilast because this cytochrome member resembles the main metabolic pathway for roflumilast (Westphal, 2000).

A little while ago, roflumilast was predicted to exert anti-viral effect similar to that of lopinavir/ritonavir via binding very close to the middle pocket of SARS-CoV-2 3CLpro and thereby, interfering with its activity (Hu et al., 2020). Then, roflumilast can deprive the virus from hydrolyzing the polyprotein into functional proteins required for its replication, **Figure 3** (He et al., 2020). However, the preventive and therapeutic effectiveness of roflumilast against COVID-19 and its pharmacological mechanisms have not been yet extensively studied.

10. NEP-based strategy for treating COVID-19 by roflumilast

One of the proposed NEP-dependent mechanisms for blocking the airway inflammation is to cleave the neutrophil-released cathepsin G, that is documented to convert both angiotensinogen and angiotensin I into angiotensin II, **(Fig. 4)** (Meyer-Hoffert, 2009; Pham, 2006; Wintroub et al., 1984).

In response to severe COVID-19 infection, ang II is reported to be continuously generated to probably lead to the systemic cytokine storm (Xiong et al., 2020). Among the released cytokines, IL-6 will play a vital role in the progression of numerous inflammatory reactions as well as endothelial dysfunction and platelet activation (Funakoshi et al., 1999; Y. Liu et al., 2020). Therefore, cleaving cathepsin G by NEP with reducing associated Ang II formation may be a logical commentary for the suppressed IL-6 expression detected following roflumilast treatment (Feng et al., 2017).

Postulating that IL-6 may be a key regulator of COVID-19 pathogenesis (T. Liu et al., 2020), decreasing its level by roflumilast will be of great importance. First, roflumilast can stop IL-6-mediated intestinal, olfactory, and ocular inflammation and consequently, inhibit the induction of anosmia, diarrhea, and conjunctivitis, respectively. Second, roflumilast may suppress the endothelial activation and inflammatory thrombocytosis prompted by IL-6 release.

As a result of the endothelial dysfunction, neutrophils trafficking has also been implicated in the pathogenesis of COVID-19, since their activation and accumulation are reported to be associated with tissue damage, exaggerated inflammation and disordered tissue repair (Tay et al., 2020). As such, NEP can degrade the chemoattractant fMLP, which was known to be involved in neutrophil chemotaxis. Hence, NEP may specifically prevent the recruitment of neutrophils across the endothelial barrier from the blood circulation into the infected tissues (Sato et al., 2013). In particular, the potential role of roflumilast in inhibiting the adhesion and transmigration of neutrophils and their subsequent inflammatory sepsis may be attributable to increased NEP activity (Hui Li et al., 2020; Sanz et al., 2007).

Additionally, NEP was reported to effectively breakdown the endothelium-derived ET-1; preventing the activation and aggregation of platelets as a result of prohibiting the synthesis of PAF (Mustafa et al., 1995; Rao and White, 1982), which was previously demonstrated to be also suppressed by the action of PDE4i (Tenor et al., 1996). Accordingly, this observation may reflect the potential NEP-dependent anti-coagulant role of roflumilast against the thromboembolic events in COVID-19; empowering it to restrain the development of PIC which is the initial step for evolving stroke in COVID-19 patients (Avula et al., 2020).

In line, it was also shown that COVID-19 patients may show pulmonary fibrosis, from which NEP may protect lungs by stopping the ET-1-induced TGF-β1, ensuring the concept that roflumilast may have the potential to attenuate the fibroblast activities and thereby, the ability to function as anti-fibrotic agent via blocking the fibrosis driven by TGF-β1 (Dunkern et al., 2007; Togo et al., 2009). Additionally, breaking ET-1 by NEP will prolong the anti-inflammatory effect of roflumilast via maintaining the high cAMP level which is underscored to play an important role in improving the immune system of highly risk COVID-19 groups (Graf et al., 1995; Raker et al., 2016).

Furthermore, enhancing NEP activity may explain the potential cardiovascular benefits of roflumilast. During the airway inflammation, NEP itself may act indirectly to decrease the blood pressure via degrading cathepsin G, that consequently inhibits the formation of angiotensin II. Decreasing angiotensin II level will direct the pulmonary renin angiotensinogen system (RAS) for generating more angiotensin (1‑7) which, via Mas receptor, can induce natriuresis/diuresis (Shah et al., 2010) and trigger the endothelial nitric oxide synthase (eNOS) to stimulate nitric oxide (NO) release, promoting blood vessel relaxation (Fraga-Silva et al., 2008; Patel and Schultz, 2013).

Accordingly, we recommend that future clinical efforts should be driven towards ensuring the NEP-mediated pharmacotherapeutic mechanisms of roflumilast proposed for counteracting COVID-19 infection.

11. Conclusion

Reducing the patient's risk of COVID-19 progression is assumed to be biologically linked with suppression of the neutrophilic component that predisposes to increased systemic inflammation and coagulopathy associated with COVID-19 infection. Therefore, management of COVID-19 should focus on modulating neutrophil function and their response. According to the underlying guidelines, recommended anti-inflammatory therapies for COVID-19 do not provide treatment satisfaction and effectiveness until now.

As the search continues, PDE4i has been suggested to offer an intriguing new class of COVID-19 treatment, since inhibiting PDE4 is thought to exhibit effective anti-inflammatory and anti-platelet activities. Among the clinically used PDE4i, roflumilast has been reported to be the most selective and effective drug submitted for treating many neutrophils-mediated airway inflammatory disorders.

Furthermore, roflumilast has been recently reported to behave as a potential inhibitor of 3CLpro, which is a proteolytic enzyme required for viral replication within the host cells.

Considering COVID-19 treatment, roflumilast may also have additive advantages to the concurrent protocol, since it had been reported to be used safely in combination with either corticosteroids, azithromycin and recommended vitamins

(C, E and Zinc) without showing any dangerous adverse effects up till now. As well, via attenuating the airway neutrophilic inflammation, roflumilast can enhance the compromised anti-inflammatory properties of corticosteroids and improve their resistance effect.

Additionally, because of increasing cAMP level, we suppose that roflumilast can prolong its anti-inflammatory effect and display other therapeutic properties via enhancing NEP activity, which is proposed to be an important target for managing COVID-19.

Therefore, taken into our consideration that this review is the first one to discuss the NEP-mediated therapeutic properties of roflumilast and its role in facing the inflammatory, coagulopathy and fibrotic cascades driven by COVID-19, we hope that our hypothesis will serve as a stimulus for further confirmation about the therapeutic impact of roflumilast in COVID-19 management and consequently, may provide physicians with a novel repurposed treatment option against COVID-19.

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Conflict of interest

The authors declare no conflict of interest. The authors and their institutions are the only responsible for the financial support and the content of this work in the submitted manuscript. All other authors have no conflict of interests to disclose.

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Table 1: Multiple pharmacological properties of roflumilast

pharmacological effect of roflumilast	Dose	Model (in vitro/ in vivo/clinical trial)	Main molecular mechanisms of action	References
Inhibition of neutrophil function	10^{-9}– 10^{-6} M	Neutrophil adhesion to HUVECs	Suppressed the release of MPO, NE and MMP-9	(Jones et al., 2005)
	1–1000 nM $L^{-}1$	Human PLTs and PMNs	Inhibited the release of NETs and suppressed tissue factor expression in MNs	(Totani et al., 2016)

Anti-inflammatory effect	500 µg/d	COPD patients	Inhibited phosphodiesterase-4 enzyme that targets the systemic inflammation associated with COPD and decreased inflammatory mediators	(Martinez et al., 2015)
	500 µg/d	Allergic asthmatic patients	Inhibited allergen-induced sputum eosinophils, neutrophils and ECP	(Bateman et al., 2016; Gauvreau et al., 2011)
Prevention of polymicrobial sepsis	0.3 – 1.0 mg/kg body	Mice with cecal ligation and puncture-induced sepsis	Reduced bacterial load, inhibited expression of pro-inflammatory cytokines mainly IL-6 and TNF-alpha and suppressed NF-κB, p38 MAPK and STAT3	(Feng et al., 2017)

	1, 10, and 100 nmol/L and 1 μmol/L dissolved in DMSO	Human ASM cells	Inhibited ECM protein deposition and thereby, airway remodeling	(Burgess et al., 2006)
Inhibition of airway remodeling	5 mg/kg/d, suspended in 2.5% polyethylene glycol 4% methylcellulose solution	BALB/c mice model of chronic asthma	Reduced the accumulation of chronic inflammatory cells, and thickening of airway epithelium	(Kumar et al., 2003)
Anti-proliferative effect	10^{-9}– 10^{-6} M	Distal human PASMCs	Attenuated cell proliferation and production of (MMP-2 and MMP-9)	(Growcott et al., 2006)

Anti- fibrotic effect	5 mg/kg/day	Bleomycin-Induced Fibrosis in mice	Antagonized metabolic effects related to pulmonary fibrosis (like alterations in the oxidative equilibrium, a strong inflammatory response and collagen synthesis activation)	(Milara et al., 2015a)
	10^{-6}– 10^{-7} M	Adult human lung fibroblast cell lines	Antagonized the profibrotic activity of fibroblasts stimulated by TGF-β1	(Togo et al., 2009)
Anti-hyperglycemic effect	500 µg/d	35–70 years patients with newly diagnosed DM type II	Enhanced secretion of intestinal GLP-1, a main incretin with potent insulinotropic effect	(Wouters et al., 2012)

HUVECs: Human umbilical vein endothelial cells; MPO: Myeloperoxidase; NE: Neutrophil elastase; MMP-9: Matrix metalloproteinase-9; PLTs: Platelets; PMNs:

Polymorphonuclear leukocytes; NETs: Neutrophil extracellular traps; MN: Monocytes; COPD: Chronic obstructive pulmonary disease; ECP: Eosinophil cationic protein; NF-κB: Nuclear factor-kappa B; MAPK: Mitogen-activated protein kinase; STAT3: Signal transducer and activator of transcription 3; ASM: Airway smooth muscle; DMSO: Dimethyl sulfoxide; ECM: Extracellular matrix; PASMCs: Pulmonary artery smooth muscle cells; TGF-β1: Tissue growth factor-beta 1; DM: Diabetes mellitus; GLP-1: glucagon like peptide-1.

Figure 1

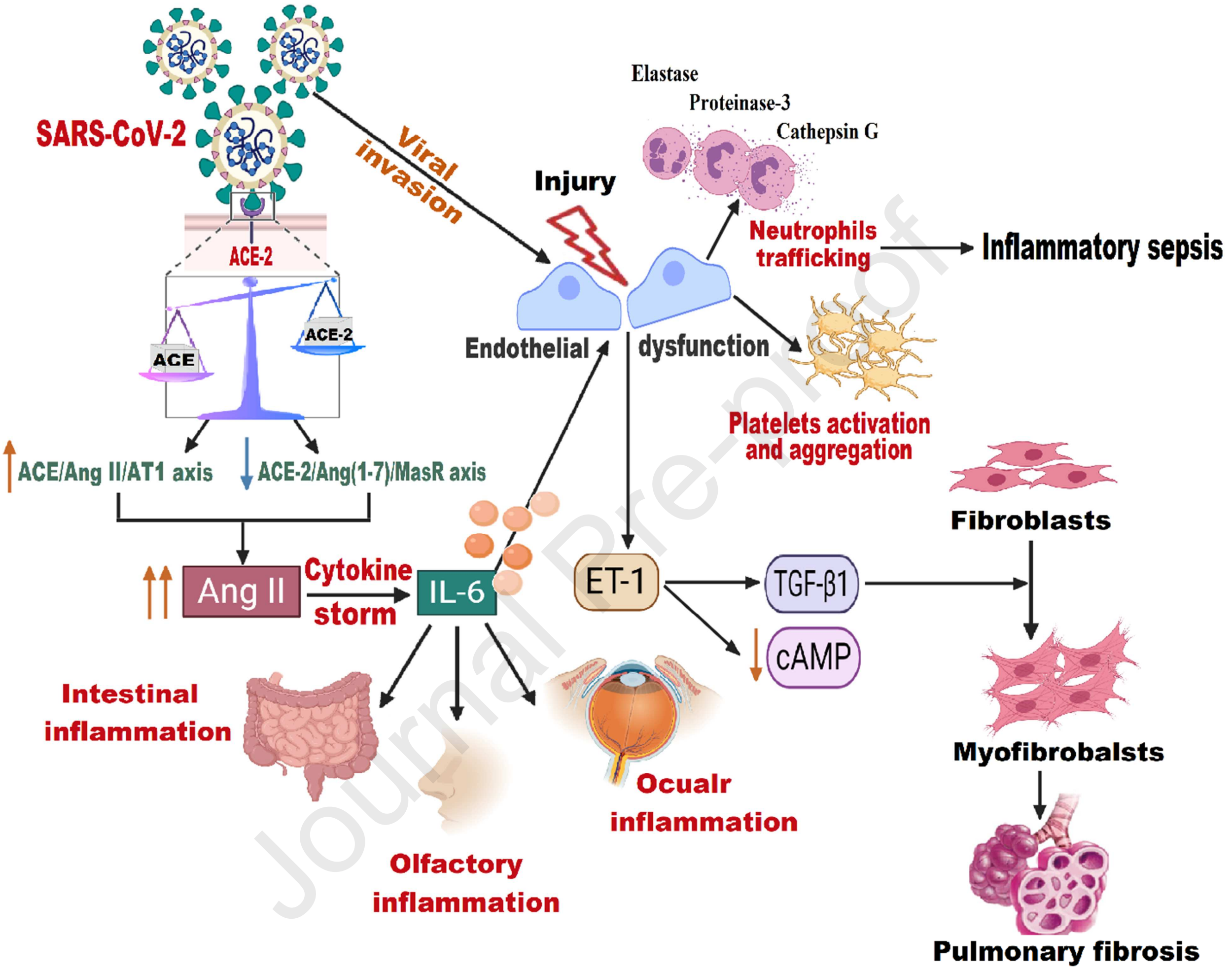
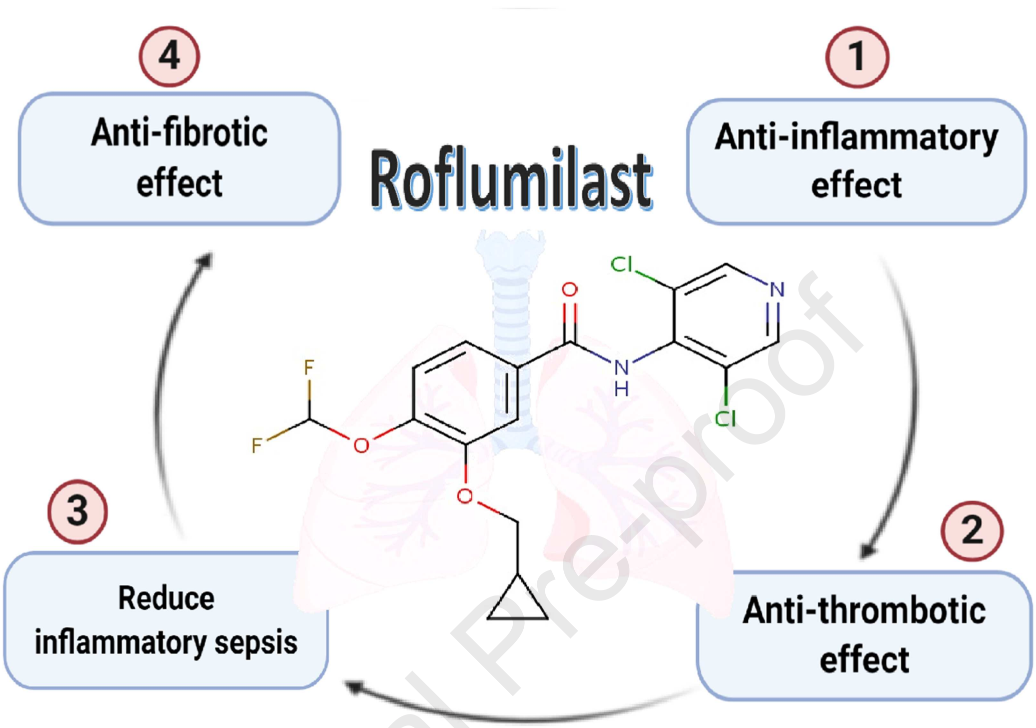

SARS-CoV-2
Viral invasion
ACE-2
ACE
ACE-2
ACE/Ang II/AT1 axis
ACE-2/Ang(1-7)/MasR axis
Ang II
Cytokine storm
IL-6
Injury
Elastase
Proteinase-3
Cathepsin G
Neutrophils trafficking
Inflammatory sepsis
Endothelial
dysfunction
Platelets activation and aggregation
Fibroblasts
ET-1
TGF-β1
cAMP
Intestinal inflammation
Olfactory inflammation
Ocualr inflammation
Myofibrobalsts
Pulmonary fibrosis

Figure 2



4
Anti-fibrotic effect
Roflumilast
1
Anti-inflammatory effect
Cl
N
O
N
H
Cl
F
F
O
O
3
Reduce inflammatory sepsis
2
Anti-thrombotic effect

Figure 3

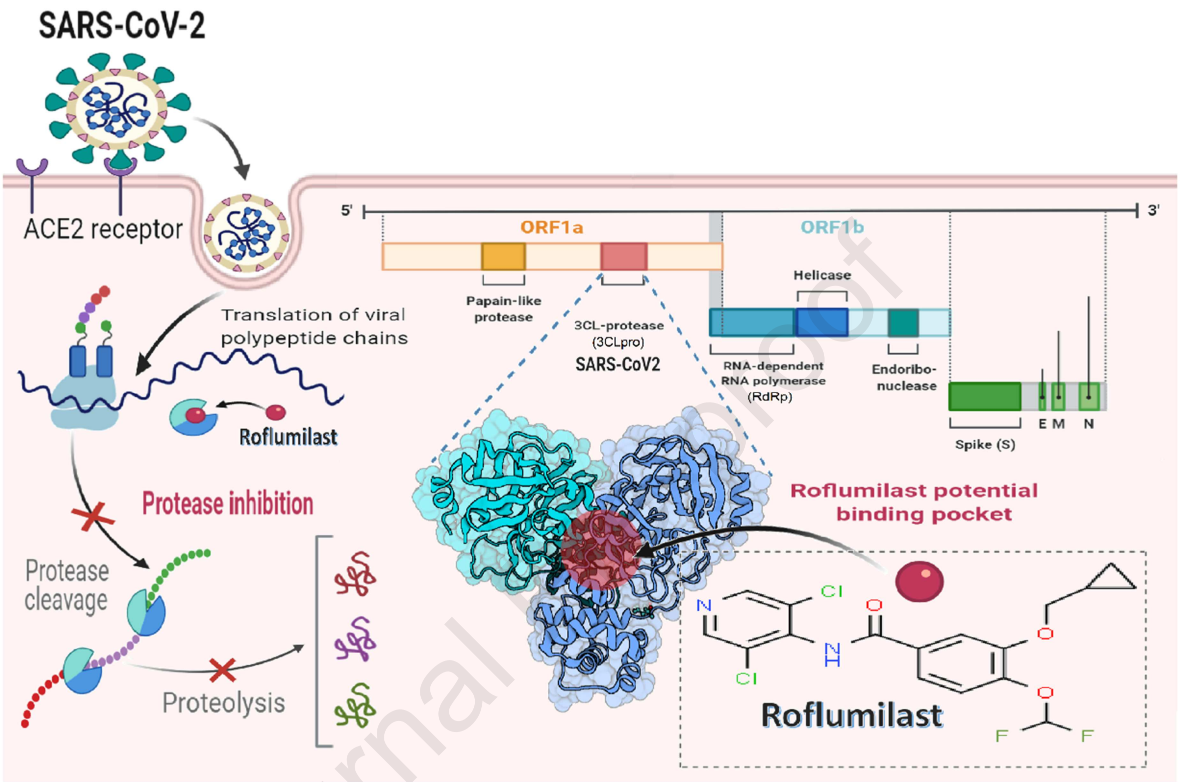
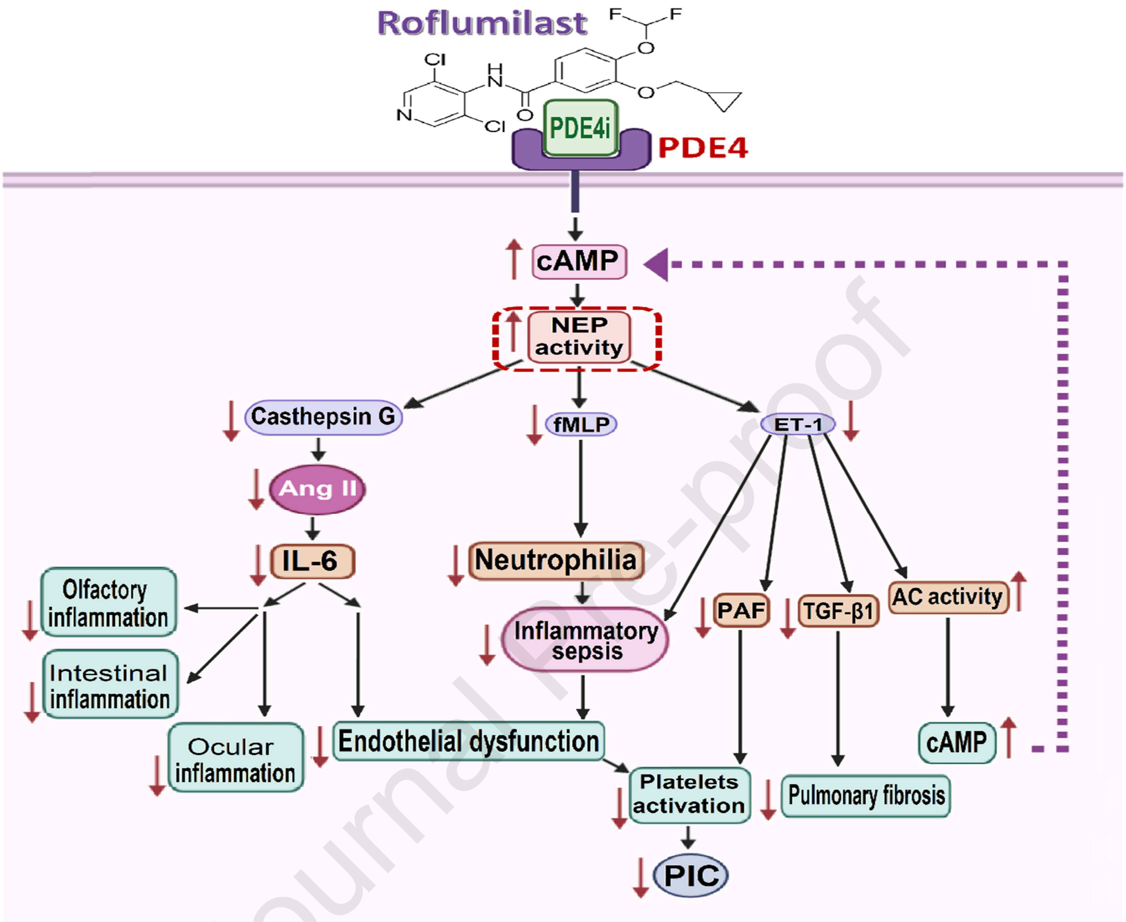

SARS-CoV-2
ACE2 receptor
Translation of viral polypeptide chains
Roflumilast
Protease inhibition
Protease cleavage
Proteolysis
5'
3'
ORF1a
ORF1b
Papain-like protease
3CL-protease (3CLpro)
SARS-CoV2
Helicase
RNA-dependent RNA polymerase (RdRp)
Endoribo-nuclease
Spike (S)
E M
N
Roflumilast potential binding pocket
Roflumilast

Figure 4



Roflumilast
PDE4i
PDE4
cAMP
NEP activity
Casthepsin G
fMLP
ET-1
Ang II
IL-6
Neutrophilia
Olfactory inflammation
Intestinal inflammation
Ocular inflammation
Inflammatory sepsis
Endothelial dysfunction
PAF
TGF-β1
AC activity
cAMP
Platelets activation
Pulmonary fibrosis
PIC

Figure 1: A schematic diagram of COVID-19 pathophysiology

Binding of Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) with angiotensin converting enzyme-2 (ACE-2) may downregulate it; inhibiting the ACE-2 / angiotensin (1–7) / Mas receptor axis and subsequently, activating the ACE / angiotensin (Ang) II / angiotensin II type 1 (AT1) receptor axis on the other side, that may lead to an increase in the level of angiotensin II. Angiotensin II could promote the release of multiple inflammatory cytokines particularly, interleukin-6 (IL-6), which could play a crucial role in inducing intestinal, olfactory and ocular inflammation, in addition to disrupting the function of endothelial cells. SARS-CoV-2 itself can also induce endothelial dysfunction; resulting in platelet activation and aggregation. Moreover, endothelial dysfunction may trigger more inflammation through trafficking more neutrophils with subsequent inflammatory sepsis. Simultaneously, secreting endothelin-1 (ET-1) as a result of endothelial dysfunction could stimulate the fibrotic consequences via persuading the release of transforming growth factor-β1 (TGF-β1), developing pulmonary fibrosis. In addition, ET-1 could also exaggerate the inflammation via decreasing the level of cyclic adenosine monophosphate (cAMP).

Figure 2: General outline of roflumilast pharmacological actions

Figure 3: Suggested anti-SARS-CoV-2 effect of roflumilast

For SARS-CoV-2 to be replicated inside the cytoplasmic membranes, its viral polyprotein chains should be firstly hydrolyzed into functional proteins either by papain like protease, 3C-like protease (3CLpro), RNA-dependent RNA polymerase (RdRp), helicase, or endoribonuclease. Roflumilast is predicted to specifically bind very close to the middle pocket of SARS-CoV-2 3CLprotease and thereby, may interfere with its proteolytic activity; preventing viral replication.

Figure 4: Proposed NEP-based therapeutic mechanisms of roflumilast in treating COVID-19

Being a highly selective phosphodiesterase-4 inhibitor (PDE4i), roflumilast acts by enhancing cyclic adenosine monophosphate (cAMP) level, which in turn will increase neprilysin (NEP) activity. Once NEP is activated, it can cleave the neutrophil-released cathepsin G and consequently, prevent angiotensin II formation. That will be accompanied by a decrease in the level of released interleukin-6 (IL-6) and its associated olfactory, intestinal and ocular inflammatory reactions as well as IL-6 -mediated endothelial dysfunction and platelet activation. Moreover, NEP can degrade the chemoattractant N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), prohibiting neutrophil recruitment and chemotaxis and hence, their subsequent inflammatory sepsis. Therefore, NEP can participate in reducing the induction of endothelial dysfunction and platelet activation. Additionally, NEP can breakdown endothelin-1 (ET-1); preventing the synthesis of platelet activating factor (PAF) and accordingly, the activation and aggregation of platelets as well as pulmonary intravascular coagulopathy (PIC) development. Degrading ET-1 can also inhibit pulmonary fibrosis via blocking the ET-1-induced transforming growth factor- β1 (TGF-β1), and at the same time, maintain the high level of cAMP which may contribute for long-term anti-inflammatory effect of roflumilast.

Highlights

- Roflumilast as a novel option for COVID-19 therapy is addressed in this review
- NEP-mediated therapeutic properties of roflumilast against COVID-19-associated inflammatory, coagulopathy and fibrotic cascades
- Roflumilast may inhibit COVID-19-induced endothelial dysfunction and coagulopathy
- Roflumilast may counteract neutrophil-mediated inflammation and subsequent sepsis in COVID-19
- Roflumilast may prevent COVID-19 prompted pulmonary fibrosis