The Counter Regulatory Axis of the Lung Renin-Angiotensin System in Severe COVID-19: Pathophysiology and Clinical Implications

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The severe acute respiratory syndrome coronavirus (SARS-CoV)-2, which is responsible for coronavirus disease 2019 (COVID-19), uses angiotensin (ANG)-converting enzyme 2 (ACE2) as the entrance receptor. Although most COVID-19 cases are mild, some are severe or critical, predominantly due to acute lung injury. It has been widely accepted that a counter regulatory renin-angiotensin system (RAS) axis including the ACE2/ANG [1-7]/Mas protects the lungs from acute lung injury. However, recent evidence suggests that the generation of protective ANG [1-7] in the lungs is predominantly mediated by proinflammatory prolyl oligopeptidase (POP), which has been repeatedly demonstrated to be involved in lung pathology. This review contends that acute lung injury in severe COVID-19 is characterised by a) ACE2 down-regulation and malfunction (inflammatory signalling) due to viral occupation, and b) dysregulation of the protective RAS axis, predominantly due to increased activity of proinflammatory POP. It follows that a reasonable treatment strategy in COVID-19-related acute lung injury would be delivering functional recombinant (r) ACE2 forms to trap the virus. Additionally, or alternatively to rACE2 delivery, the potential benefits resulting from lowering POP activity should also be explored. These treatment strategies deserve further investigation.

Keywords   Coronavirus disease 2019 • Renin-angiotensin system • Acute lung injury

Introduction

An outbreak of coronavirus disease 2019 (COVID-19) occurred in December 2019 due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is taxonomically a strain of SARS-CoV. COVID-19 was first discovered in China and then rapidly spread worldwide [1]. Observational studies reported older age and the presence of comorbidities as risk factors for increased disease severity in COVID-19 [2]. However, it soon became clear that severe COVID-19 could also occur in younger patients with or without pre-existing medical conditions [3]. The pathophysiology of severe COVID-19 is dominated by the presence of acute lung injury [4,5]. Higher levels of inflammatory markers in blood, an increased neutrophil-to-lymphocyte ratio and increased serum levels of several inflammatory
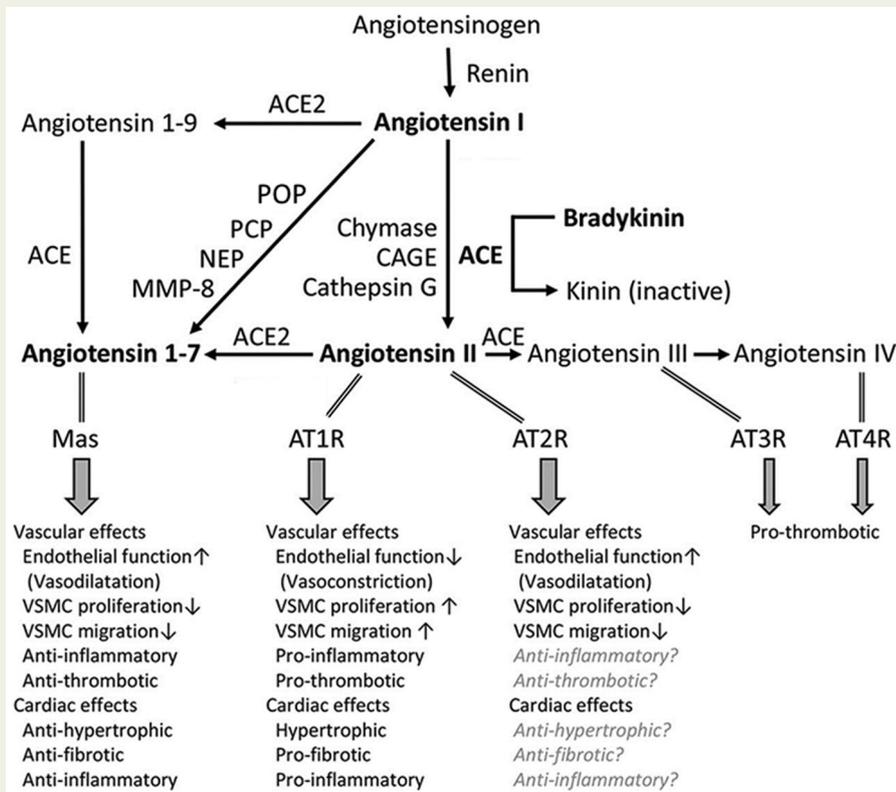
Figure 1 RAS activation.

Angiotensin II and AT1R are key molecules of the major deleterious pathway in the cardiovascular system. Counteracting pathways with a cardiovascular protective property also exist. There are controversies about the effects of AT2R signalling, which are indicated by grey italic characters.

Abbreviations: ARB, angiotensin receptor blocker; AT1R, angiotensin type one receptor; AT2R, angiotensin type two receptor; AT3R, angiotensin type three receptor; AT4R, angiotensin type four receptor; CAGE, chymostatin-sensitive angiotensin-II-generating enzyme; Mas, Mas receptor; MMP-8, matrix metalloproteinase-8; NEP, neutral endopeptidase; PCP, prolyl carboxypeptidase; POP, prolyl oligopeptidase; RAS, renin-angiotensin system; VSMC, vascular smooth muscle cell. Otto CM. Heart. 2017;103:1305–07 [10].

cytokines and chemokines have been associated with acute lung injury and mortality [6]. It has been widely accepted that a counter-regulatory renin-angiotensin system (RAS) axis including angiotensin (ANG)-converting enzyme 2 (ACE2)/ANG [1-7]/Mas protects the lungs from acute lung injury. However, recent evidence suggests that ACE2 expression is significantly lower in the lungs compared with other organs and tissues [7] and that generation of the protective ANG [1-7] is predominantly mediated by prolyl oligopeptidase (POP), which has repeatedly been demonstrated to be involved in human pathology [8].

This review contends that acute lung injury in severe COVID-19 is characterised by a) ACE2 downregulation and malfunction (inflammatory signalling) due to viral occupation, and b) dysregulation of the protective RAS axis, predominantly due to increased activity of proinflammatory POP. It follows that a reasonable treatment strategy in COVID-19-related acute lung injury deserving further investigation would be delivering functional recombinant (r) ACE2 forms to trap the virus. Additionally, or alternatively to rACE2 delivery, the potential benefits resulting from lowering POP activity should also be explored.

Renin Angiotensin System

Overview

Current understanding of the RAS has greatly evolved from the classical renin/ACE/ ANG II/ANG type 1 (AT1) receptor and AT2 receptor axis and its physiological roles in the regulation of cardiovascular and renal function, blood pressure control, aldosterone biosynthesis and release, and salt and fluid homeostasis [9]. It is now recognised that the classical renin/ACE/ANG II/AT1 and AT2 axis is no longer the exclusive effector and signalling pathway for the system [10] (Figure 1). One (1) of the new axes that have recently been described is the ACE2/ANG [1-7]/Mas axis, which counteracts or modulates the effects of the classical axis [11]. However, recent evidence suggests that although this axis may be active in other organs, this may not be the case in the lungs.
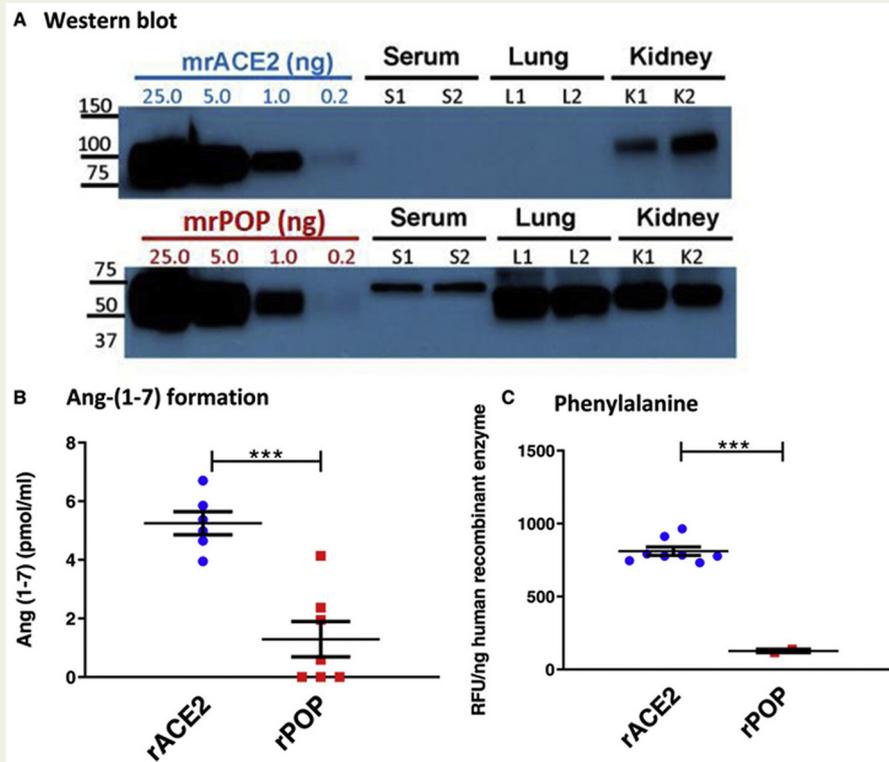
Figure 2 Angiotensin-converting enzyme 2 (ACE2) and prolyl oligopeptidase (POP) proteins in wild-type (WT) serum, lung and kidneys, and the effect of rACE2 and rPOP on Ang-(1-7) and phenylalanine formation.
A. ACE2 and POP proteins in WT serum, lung and kidneys by Western blot. ACE2 protein is abundant in kidneys, but not in serum or lungs. POP protein is present in serum, lungs and kidneys.
B. In vitro Ang II (angiotensin II) to Ang-(1-7) conversion assessed by equivalent amounts of recombinant (r) ACE2 and rPOP is higher with rACE2 than with rPOP (***$p<0.001$).
C. Generation of free phenylalanine (Phe) from Ang II as a substrate by equivalent amounts of recombinant (r) ACE2 and rPOP is higher with rACE2 than with rPOP (***$p<0.001$). For Western blot, different amounts of mouse recombinant protein standards were loaded to estimate ACE2 and POP protein expression levels in serum (1 μL), lung and kidney lysates (50 μg total protein) from two WT mice.
Serfozo P, et al. Hypertension. 2020;75(1):173–82 [15].

Synthesis of ANG [1-7] in the Lungs

The main enzymes involved in the production of ANG [1-7] from ANG I are thimet oligopeptidase (THOP), neutral endopeptidase (NEP) and POP [11,12]. In addition, ACE2, POP and prolylcarboxypeptidase (PCP) can generate ANG [1-7] from ANG II. The formation of ANG [1-7] from ANG I by ACE2 involves production of the intermediate ANG [1-9] and its subsequent cleavage by ACE or NEP. However, the catalytic efficiency of this pathway is much lower than that of the ACE2-dependent conversion of ANG II to ANG [1-7].

The degree of expression and biological relevance of ACE2 may vary depending on the tissue and clinical state [13,14]. The mRNA and protein expression of ACE2 in the lungs is significantly lower compared with other organs and tissues [7]. Moreover, a recent study that examined the relative contribution to ANG [1-7] formation from ANG II by POP and ACE2 ex vivo in serum, kidney and lung tissues demonstrated that POP is the main enzyme responsible for ANG II conversion to ANG [1-7] in the circulation and lungs,

whereas ANG [1-7] formation in the kidney is mainly ACE2-dependent (Figure 2) [15]. This study also showed that POP is less effective in converting the deleterious ANG II to the protective ANG [1-7] compared with ACE2 (Figure 2) [15].

Prolyl Oligopeptidase

Prolyl oligopeptidase is an oligopeptidase with endopeptidase activity. It has been shown to be localised in the cytoplasm but given its ability to inactivate several neuropeptides in vitro by limited proteolysis, its involvement in the in vivo generation of immunoactive peptides and its presence in plasma it most likely also has an extracellular role [8,16]. Membrane-bound POP has also been described [17]. Peptides up to 30 amino acids long that contain a proline are potential substrates of POP; examples of substrates are substance P, ANGI, ANG II, and bradykinin.

However, POP is proinflammatory as it also contributes to the generation of the matrikine proline-glycine-proline (PGP) from collagen fragments, which has classically been described
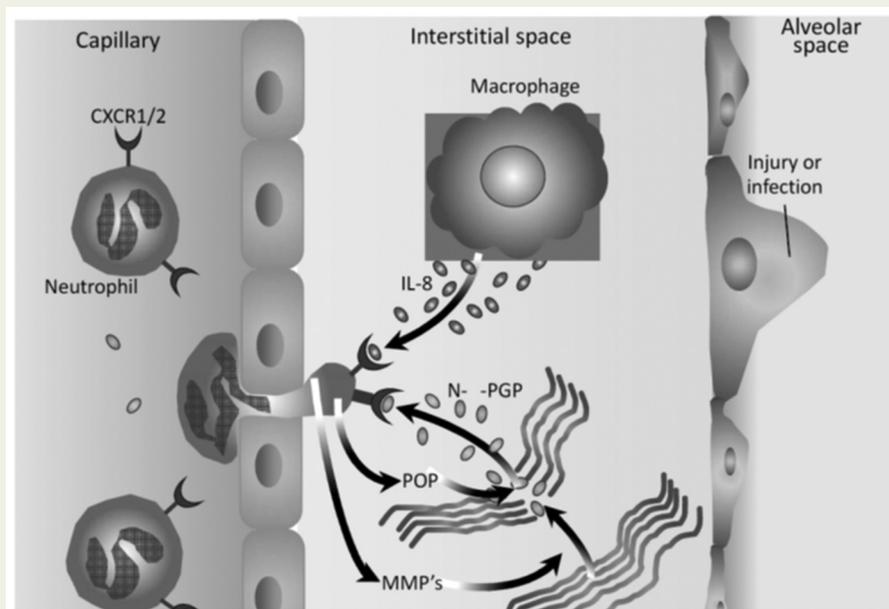
Figure 3 The generation of N-acetyl-prolyl-glycyl-proline (N-α-PGP), a product of prolyl oligopeptidase (POP) after injury or infectious insult. Activated neutrophils secrete metalloproteinases (MMPs) and POP. MMP-8 and MMP-9 cleave collagen to suitable fragments for POP cleavage to generate PGP. The PGP generated is a chemoattractant for neutrophils. Penttinen A, et al. CNS Neurol Disord Drug Targets. 2011;10:340–8 [24].

as a neutrophil chemoattractant [18]. Generation of PGP from collagen is a multistep enzymatic process that requires the concerted action of specific matrix metalloproteinases (MMPs) and POP. The initial cleavage of native collagen by MMPs yields fragments that are a suitable substrate size for POP, which subsequently acts to liberate PGP. PGP undergoes acetylation and is converted to AcPGP, which is 4–7-fold more potent [19]. MMPs can be derived from a variety of cellular sources, whilst POP has been reported to be expressed by neutrophils [20], airway macrophages [21] and epithelial cells [21,22]. Since neutrophils are an abundant source of the proteases that generate PGP, it is anticipated that this pathway can drive a self-sustaining cycle of neutrophilic inflammation [23,24] (Figure 3), which links POP to the pathology of many chronic lung diseases [22].

Mas

Evidence for a specific binding site for ANG [1-7] was demonstrated in 2003 with the finding that Mas is a receptor for heptapeptide [25]. Nevertheless, ANG [1-7] has no effect on Mas-transfected cells but exerts biased agonism or even antagonism at the ANG type-1 receptor (AT1R) [26]. Moreover, biased signalling of Mas itself has been described and heteromeric interactions of Mas with AT1R and AT2R, bradykinin B2 and endothelin B receptors [26]. Therefore, future studies need to clarify the relationship between Mas and ANG [1-7].

In conclusion, POP is the main enzyme responsible for the production of ANG [1-7] in the lungs. However, POP less effectively converts the deleterious ANGII to the protective ANG [1-7] and is proinflammatory.

ACE2 and Coronavirus Entry

ACE2 has been demonstrated as the receptor of entry for SARS-CoV and for SARS-CoV-2 [27]. The major entry site of SARS-CoV-2 is via the respiratory system. The type II transmembrane serine proteases TMPRSS2 (Transmembrane Protease Serine 2) and ADAM17 (A Disintegrin and Metalloproteinase) promote SARS-CoV-2 entry by two separate mechanisms: ACE2 cleavage, which might promote viral uptake, and SARS-CoV-2 Spike cleavage, which activates the Spike protein for membrane fusion [28]. A defined receptor-binding domain of SARS-CoV-2 Spike specifically recognises its host receptor ACE2, and host susceptibility to SARS-CoV-2 infection is primarily determined by the affinity between the viral binding domain and host ACE2 in the initial phase of the disease [29]. The attachment of the viruses to cell surface ACE2 protects them from immune surveillance mechanisms, leaving them tagged to the host for relatively longer periods, thus making them efficient carriers and vulnerable hosts for future infections and spread [30]. In addition to the full-length transmembranous form, ACE2 also exists in soluble form (sACE2) in the circulation. The latter results from ADAM17-mediated ectodomain "shedding" from endothelial cells into the circulation, lacks the membranous anchor and may act as the bait to neutralise the Spike protein on the surface of SARS-CoV-2 [31].

After viral Spike protein binding to the cellular receptor ACE2, the conformation change in the Spike protein facilitates viral envelope fusion with the cell membrane through the endosomal pathway [32]. ACE2 occupied with SARS-CoV-2 is engaged in inflammatory signalling. The expression of ACE2, SARS-CoV Spike protein and some proinflammatory cytokines in autopsy tissues from patients who died of SARS were studied with immunohistochemistry and in situ hybridisation assays [33]. SARS-CoV Spike protein and its RNA were only detected in ACE2+ cells in the lungs and other organs, indicating that ACE2-expressing cells are the primary targets for SARS-CoV infection in vivo in humans. High levels of proinflammatory cytokines were expressed in the SARS-CoV-infected ACE2+ cells, but not in the uninfected cells, suggesting that cells infected by SARS-CoV produce elevated levels of proinflammatory cytokines, which may cause immunomediated damage to the lungs, resulting in acute lung injury [33]. The findings in another study were similar [34], which showed that the high expression of ACE2 is related to innate immune responses, adaptive immune responses, B cell regulation, cytokine secretion, and an enhanced inflammatory response induced by IL-1, IL-10, IL-6, and IL-8 [34].

Pathogenesis of Severe COVID-19

Most of the patients with COVID-19 who become critically ill tend to have minor symptoms in the early stages of the disease. The condition of these patients suddenly deteriorates in the later stages of the disease or in the process of recovery, usually due to acute lung injury [35]. Although the mechanisms of COVID-19-induced acute lung injury are still being investigated, the term cytokine storm syndrome, which is characterised by an uncontrolled activation and proliferation of T lymphocytes and macrophages, has become synonymous with its pathophysiology [36]. Based on this presumption, dexamethasone [37] and colchicine [38] have been tested in severe COVID-19 with encouraging initial results in selected patients.

However, increased levels of plasma biomarkers – including markers of systemic inflammation, epithelial injury and dysregulated coagulation – are frequently observed in acute lung injury, regardless of aetiology [39]. Moreover, as the median IL-6 levels in patients with the hyperinflammatory phenotype of acute respiratory distress syndrome (ARDS) are 10-fold to 200-fold higher than levels in patients with severe COVID-19, it seems that severe viral pneumonia from COVID-19 primarily produces severe lung injury, without the same magnitude of systemic responses in most patients with COVID-19 [40]. This has been corroborated by the findings of a recent post-mortem study of patients with COVID-19 ARDS, which reported that severe vascular injury, including alveolar microthrombi, was nine times more prevalent than that found in post-mortem studies of patients with influenza ARDS [41].

Based on the above it is evident that the two major components of acute lung injury in severe COVID-19 are inflammatory and thrombotic. Development of both components is compatible with downregulation and malfunction (inflammatory signalling) of the virus-occupied ACE2 as well as dysregulation of the counter-regulatory RAS axis in the lungs, predominantly due to increased activity of proinflammatory POP.

ACE2 and Acute Lung Injury

ACE2 downregulation has been implicated in diverse models of acute lung injury by inducing an imbalance in the RAS. It has been proposed that in acute lung injury: (i) a decrease in pulmonary ACE2 and increase in ANG II levels occur; (ii) supplementation with ACE2 or inhibition of ANG II improves outcomes; and (iii) a lack or decrease of pulmonary ACE2 aggravates viral-induced acute lung injury [42]. These conclusions are predominantly based on experimental studies evaluating pulmonary involvement in the setting of pulmonary hypertension and fibrosis [43,44], bleomycin-induced lung injury [45], smoke inhalation-induced ARDS [46], and lipopolysaccharide-induced lung injury in piglets [47]. However, a decrease in ACE2 was also observed in the mouse model of acid aspiration-induced acute lung injury, in which injection of SARS-CoV Spike further worsened acute lung failure, which could be attenuated by blocking the renin-angiotensin pathway with losartan [48]. Shedding of ACE2 upon binding of the viral Spike protein seems to be responsible for the markedly reduced ACE2 expression in the context of SARS-CoV infection; however, trapping and subsequent degradation of ACE2 in the constitutive secretory pathway of infected cells might also contribute to this [49].

Thus, ACE2 downregulation is a universal finding in acute lung injury, regardless of the underlying cause, and favours the progression of inflammatory and thrombotic processes triggered by local ANG II hyperactivity. Specifically, in acute lung injury in the COVID-19 setting, ACE2 is additionally occupied by SARS-CoV-2 and is engaged in inflammatory signalling.

POP and Acute Lung Injury

POP levels, in contrast to ACE2, increase in acute lung injury. Intrathecal administration of lipopolysaccharide in mice has been found to induce the release of POP containing exosomes [22]. Neutrophilic airway inflammation induced by cigarette smoke exposure in mice was associated with elevation of POP activity and PGP levels [21]. POP was highly expressed in the epithelial and inflammatory cells (macrophages and neutrophils) of lung tissue. After cigarette smoke exposure removal, neutrophil influx, POP activity and PGP levels decreased or returned to normal. Neutrophil influx was also significantly decreased in bronchoalveolar lavage fluid after administration of L-arginine-threonine-arginine, (RTR), an inhibitor of PGP that is synthesised by the action of POP, indicating the importance of POP and PGP in neutrophil migration to the lungs [21]. In the same study the increase in POP expression

was attributed to inflammatory cell influx in the lungs, since the inflammatory cells (macrophages and neutrophils) highly express POP. However, the authors did not exclude the possibility that POP could also be released as a result of airway epithelial necrosis or necrosis of other inflammatory cells. Likewise, in mice, in which *Pseudomonas aeruginosa*-derived lipopolysaccharide was intratracheally instilled to induce acute lung injury, pre-treatment with RTR significantly inhibited lipopolysaccharide-induced acute lung injury by attenuating lung neutrophil infiltration, pulmonary permeability and parenchymal inflammation [50]. Finally, another experimental study provided evidence that PGP may be important in the pathogenesis of chronic obstructive pulmonary disease (COPD), especially when disease progress is related to neutrophilic inflammation, and that administration of RTR may prevent or ameliorate the clinical manifestations of COPD [51].

Rosmarinic acid is a natural POP inhibitor, which increases superoxide dismutase activity, suppresses ERK/MAPK signalling and inhibits complement cascade [52]. In mice with acute lung injury induced by lipopolysaccharide, rosmarinic acid significantly decreased the production of lipopolysaccharide-induced TNF-α, IL-6 and IL-1β [53]. Moreover, rosmarinic acid significantly decreased the lung wet-to-dry weight ratio as well as the number of total cells, neutrophils and macrophages in the bronchoalveolar lavage fluid. Finally, rosmarinic acid exerted strong anti-inflammatory and antioxidative effects and induced amelioration of lung pathological insults comparable to that of dexamethasone in sensitised and asthmatic rats [54–56].

Roflumilast is a phosphodiesterase 4 inhibitor (PDE4i) used as add-on therapy to suppress inflammation for a subgroup of patients with severe COPD and persistent symptoms or exacerbations, despite optimal management [57]. Roflumilast reduces the number of neutrophils and eosinophils in induced sputum of patients with COPD [58]. Also, the levels of soluble interleukin-8, neutrophil elastase, eosinophil cationic protein, and α2-macroglobulin are significantly reduced with roflumilast [59]. Although the detailed mechanism of how roflumilast suppresses inflammation in COPD patients is not well understood, the self-propagating AcPGP pathway is involved and it seems that the mechanism with which roflumilast reduces pulmonary inflammation includes lowering POP activity [60]. Roflumilast has proven to be effective in experimental models of acute lung injury, including inhalation of lipopolysaccharide [61], repetitive saline lung lavage [62] and pulmonary air embolism [63].

Thus, there is evidence suggesting that increased POP activity regularly occurs in acute lung injury. Most importantly, it seems that POP itself contributes to acute lung injury development, as indicated by the beneficial effects resulting from lowering its activity. Therefore, it is likely that both ACE2 downregulation and malfunction as well as increased activity of proinflammatory POP contribute to the severity of COVID-19 (Figure 4).

Therapeutic Implications

Altering the ACE2 and POP lung levels may prove to be beneficial in the management of severe acute lung injury due to COVID-19.

Delivery of Soluble Form of ACE2

Soluble recombinant ACE2 (srACE2) prevents lung injury not only by neutralising the virus but also by releasing cellular ACE2 and enhancing its activity [64,65]. Human srACE2 has been found to reduce SARS-CoV-2 recovery from Vero cells by a factor of 1,000–5,000, whereas an equivalent mouse srACE2 had no effect. Moreover, human srACE2 S inhibited infection by SARS-CoV-2 of engineered human blood vessel organoids and human kidney organoids [66]. Beneficial effects of exogenous rACE2 protein injection have also been observed in severe acute lung injury and acute ANG II-induced hypertension [67–69]. Moreover, the administration of rACE2 in mouse models has also been shown to inhibit myocardial remodelling and attenuate ANG II-induced cardiac hypertrophy and cardiac dysfunction [70], as well as renal oxidative stress, inflammation and fibrosis [71,72].

The encouraging results of experimental studies are corroborated by studies in humans, which have demonstrated that rACE2 is safe, with no adverse effects in patients with acute lung injury and healthy volunteers [73,74]. It is noteworthy that the results of a pilot study showed that the infusion of rhACE2 tends to decrease the interleukin-6 (IL-6) concentration in patients with ARDS [73]. A limitation of both human and mice rACE2 is that it exhibits a fast clearance rate, with a half-life of only hours. Recently, a fusion protein consisting of murine rACE2 with a Fc fragment (rACE2-Fc) showed long-lasting effects [75]. Findings in a recent study were similar, which used a recombinant protein generated by connecting the extracellular domain of human ACE2 to the Fc region of the human immunoglobulin IgG1 and a fusion protein containing an ACE2 mutant with low catalytic activity [76]. Both fusion proteins demonstrated a high binding affinity for the receptor-binding domains of SARS-CoV and SARS-CoV-2. Thus, rACE2 exhibiting desirable pharmacological properties have been constructed, which may have potential applications in the diagnosis, prophylaxis and treatment of SARS-CoV-2.

Lowering of POP Activity

Experimental data indicate that lowering of POP activity effectively protects the lungs against oxidative stress and inflammation damage of diverse aetiology, and may serve as a useful treatment modality in severe COVID-19 by suppressing airway inflammation and oxidative stress, and subsequently preventing acute lung injury. Admittedly, enzymes like POP are "catalytic machines" performing chemical reactions on substrates. Evolution optimised the speed of enzyme reactions, but excessive enzyme production under
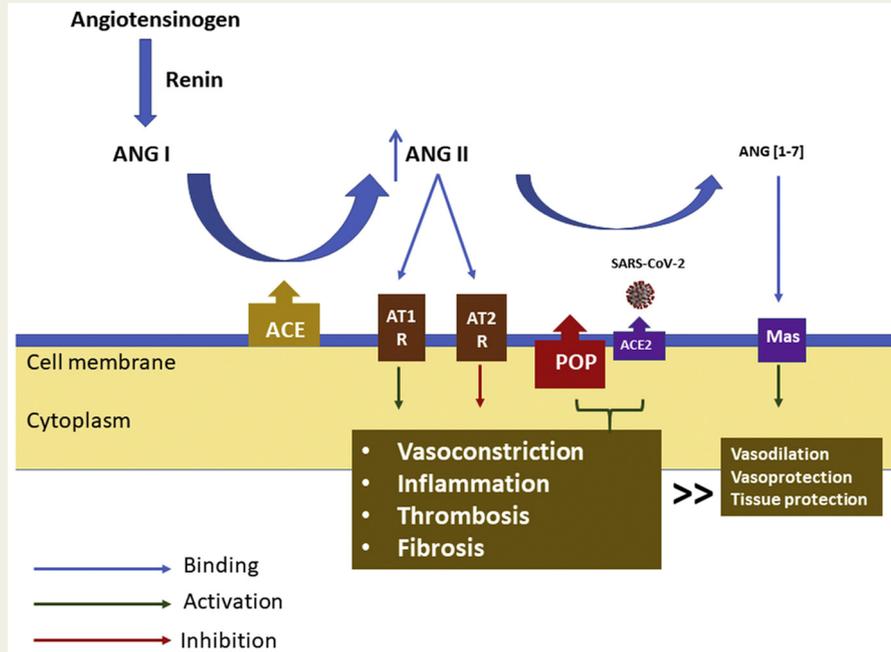
Figure 4 Presumed mechanism of acute lung injury in coronavirus disease 2019 (COVID-19) and therapeutic implications. Angiotensin-converting enzyme 2 (ACE2) serves as the severe acute respiratory syndrome coronavirus (SARS-CoV)-2 receptor. Moreover, compared with ACE2, proinflammatory prolyl oligopeptidase (POP) less effectively converts the deleterious angiotensin (ANG) II to protective ANG [1-7]. COVID-19 is associated by a downregulation and malfunction (inflammatory signalling) of ACE2 and increased POP activity. As a result, deleterious signalling (inflammation, thrombosis and fibrosis) dominates overprotective (vasodilation, vasoprotection and tissue protection) signalling in patients with severe COVID-19, culminating in acute lung injury. Lung delivery of recombinant ACE2 retaining its functionality and/or lowering of POP activity are reasonable treatment modalities that deserve further testing.

Abbreviations: AT1R, angiotensin receptor type 1; AT2R, angiotensin receptor type 2.

pathologic conditions could lead to non-controlled and accelerated activity, which must be blocked to avoid a product that promotes disease. As a result, many inhibitors of enzymatic activity became drugs blocking the production of the aberrant product. However, most enzymes have several substrates, and inhibitors may block the other substrates too. It is encouraging that the available PDE4 inhibitors also lower POP activity and generally appear to be well tolerated; some of them have been tested in humans and one (roflumilast) is included in the guidelines for the anti-inflammatory treatment of COPD [58,77].

Conclusion

The devastating complications of COVID-19 are related to a hyperinflammatory and thrombotic response culminating in acute lung injury. The specific underlying mechanism(s) still need(s) to be further clarified, and a deeper understanding of the pathogenesis will result in better management and an improvement in outcome. However, there is evidence to suggest that inflammatory signalling is initiated by the binding of SARS-CoV-2 with the ACE2 receptor and is most likely reinforced by the proinflammatory POP, the substitute for ACE2 in the conversion of the deleterious ANG II to the

protective ANG [1-7] in the lungs. As ACE2 facilitates coronavirus entry and POP is proinflammatory, both have deleterious effects contributing to the development of hyperinflammation and thrombosis in severe COVID-19. It is speculated that restoration of lung ACE2 levels with rACE2, which retains the functional properties of ACE2 but additionally traps SARS-CoV-2, may become one of the most promising approaches for future treatment of patients with COVID-19. A reasonable add-on or alternative strategy would be the targeted lowering of lung POP activity, which has been proven effective in experimental models of acute lung injury as well as in human studies including patients with severe COPD.

Funding Sources

Declaration of Interest

Conflict of Interest

There are no conflicts of interest to disclose.

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