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Review

Review

Foe and friend in the COVID-19-associated acute kidney injury: an insight on intrarenal renin-angiotensin system

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Abstract

Since the first reported case in December of 2019, the coronavirus disease 2019 (COVID-19) has became an international public health emergency. So far, there are more than 228,206,384 confirmed cases including 4,687,066 deaths. Kidney with high expression of angiotensin-converting enzyme 2 (ACE2) is one of the extrapulmonary target organs affected in patients with COVID-19. Acute kidney injury (AKI) is one of the independent risk factors for the death of COVID-19 patients. The imbalance between ACE2-Ang(1-7)-MasR and ACE-Ang II-AT1R axis in the kidney may contribute to COVID-19-associated AKI. Although series of research have shown the inconsistent effects of multiple common RAS inhibitors on ACE2 expression and enzyme activity, most of the retrospective cohort studies indicated the safety and protective effects of ACEI/ARB in COVID-19 patients. This review article highlights the current knowledge on the possible involvement of intrarenal RAS in COVID-19-associated AKI with a primary focus on the opposing effects of ACE2-Ang(1-7)-MasR and ACE-Ang II-AT1R signaling in the kidney. Human recombinant soluble ACE2 or ACE2 variants with preserved ACE2-enzymatic activity may be the best options to improve COVID-19-associated AKI.

Key words COVID-19, acute kidney injury, intrarenal renin-angiotensin system, angiotensin-converting enzyme 2

Introduction

Since December 2019, the coronavirus disease 2019 (COVID-19) has been known as an acute infectious disease of the respiratory tract and recognized as an international and widespread public health emergency (https://covid19.who.int). COVID-19 is caused by a recently identified coronavirus, severe acute respiratory syndrome coronavirus (SARS-CoV)-2. Similar to SARS-CoV, SARS-CoV-2 can bind to angiotensin-converting enzyme 2 (ACE2) via its spike protein controlling infection and cell entry [1,2]. The binding of SARS-CoV-2 and ACE2 may result in the down-regulation of membrane-bound ACE2 level, thereby contributing to the diffusion of alveolar injury, acute respiratory failure, and multi-organ dysfunction, including acute kidney injury (AKI) [3]. However, it is still a hypothesis that lacks direct evidence, and further studies are needed to clarify it. On the other hand, ACE2 has received substantial attention in recent years as an essential component of the non-classic renin-angiotensin system (RAS) that regulates internal balance with an opposite function to that of angiotensin-converting enzyme (ACE) [4]. ACE inhibitor (ACEI) and angiotensin receptor blocker (ARB) can influence ACE2 expression/activity in multiple tissues, indicating that these drugs may affect COVID-19 prognosis or outcome [5–7]. However, the data published so far on RAS inhibitors' effects on the clinical outcomes of COVID-19 patients are still inconsistent.

RAS has been known for nearly 120 years. Tigerstedt and Bergman first reported a pressor substance in the crude saline extracts of the kidney and named it renin in 1898 [8]. The activation of classic and systemic RAS requires multiple steps including two cleavage processes with multi-organ interaction. First, angiotensinogen (AGT) is mainly produced by the liver and cleaved into angiotensin (Ang) I by renin, which is released from the juxtaglomerular apparatus and collecting ducts in the kidney. Second, Ang I is cleaved by ACE and become activated Ang II. Third, the Ang II binds to Ang II type 1 receptor (AT1R) to induce multiple effects, including vasoconstriction, hypertension, and vascular remodeling. In addition to classic RAS defined as the ACE-Ang II-AT1R axis, the ACE2-Ang

(1-7)-Mas receptor (MasR) cascade is known as a non-classic RAS [4]. Ang(1-7) is generated through the following pathways: the proteolysis of Ang II by ACE2, or Ang I, is converted to non-activated Ang(1-9) by ACE2 and then converted to Ang(1-7) by ACE. These Ang(1-7) peptides bind to the MasR to exhibit cardiovascular protective effects, opposing the ACE-Ang II-AT1R axis. The Yin and Yang of ACE-Ang II-AT1R and ACE2-Ang(1-7)-MasR pathways (Figure 1) interact with each other, contributing to the maintenance of blood pressure and internal environment [4,9,10].

In addition to the above well-established systemic RAS, strong evidence suggests a local RAS in the local organs/tissues, including the brain, heart, vessels, adipose, and kidney [11]. Multiple RAS components, including ACE, ACE2, renin, AGT, AT1R, and MasR, have been found in the renal tubules [12,13]. Aberrant activation of intrarenal RAS has been recognized as a critical mechanism for the pathogenesis of hypertension and renal disease [12,13]. It is well-known that ACE2-Ang(1-7)-MasR axis functions as an endogenous counterregulatory arm to the ACE-Ang II-AT1R axis in the critically ill [14]. This article focuses on recent progress on the possible involvement of intrarenal RAS, ACE-Ang II-AT1R, and ACE2-Ang(1-7)-MasR axis, in COVID-19-related AKI.

COVID-19 and Acute Kidney Injury

In addition to acute lung injury and respiratory failure, the kidney may be an essential target organ for SARS-CoV-2 infection and invasion because ACE2 exceptionally presents in proximal tubular epithelial cells of kidneys [15,16]. Several clinical studies indicated that AKI was an uncommon complication in COVID-19 patients [17–19]. Some other reports have shown that AKI worsens prognosis [20] and carries high in-hospital mortality [19] in patients with COVID-19. AKI is common among patients hospitalized with COVID-19 [21,22] and can be a severe complication of COVID-19 [23]. Indeed, the incidence of AKI was about 3%–15% in regular patients infected with COVID-19, 14.5%–50% in patients with severe COVID-19 infection in the Intensive Care Unit, and even higher in patients with chronic kidney disease, which is related to severe infection and higher fatality rate in COVID-19 patients [18,22,24–29]. More importantly, increasing evidence has shown that AKI is one of the independent risk factors for the death of critically ill COVID-19 patients [27,30]. COVID-19 patients with AKI exhibited proteinuria and hematuria, diffuse acute proximal tubular injury with cytoplasmic vacuoles, severe collapsing focal glomerulopathy, glomerular ischemia, and endothelial cell injury [29,31–40]. Although an earlier study by Wang *et al.* [41] showed COVID-19-related AKI was likely to be related to multi-organ failure but not the kidney tropism of SARS-CoV-2, Braun *et al.* [42] recently reported that SARS-CoV-2 renal tropism is associated with disease severity and development of AKI. The variation in these reports

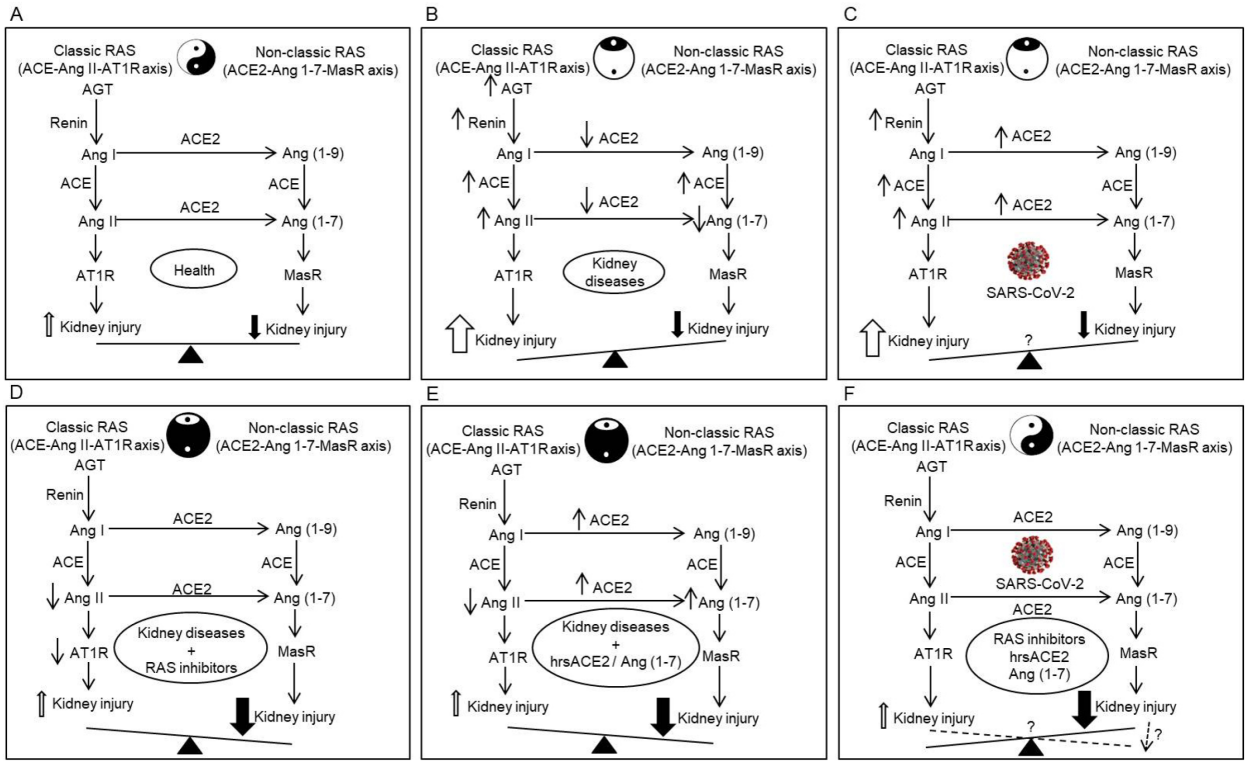

Figure 1. The schematic diagram of the ACE-Ang II-AT1R axis and ACE2-Ang(1-7)-MasR axis acting in a Yin–Yang relationship (A) In health, RAS is in balance, and no disease develops. (B) With perturbations from kidney diseases, there is a shift towards the ACE/Ang II pathway and away from the ACE2/Ang(1-7) pathway. (C) SARS-CoV-2 infection stimulates a shift towards the ACE/Ang II pathway to propagate acute kidney injury in COVID-19 patients. (D) In patients with kidney diseases, ACEI and ARB block the ACE/Ang II pathway and shift the RAS towards the ACE2/Ang(1-7) pathway. (E) In patients with kidney diseases, hrsACE2 and Ang(1-7) block the ACE/Ang II pathway and shift the RAS towards the ACE2/Ang(1-7) pathway. (F) In patients with COVID-19, ACEI and ARBs' therapy or hrsACE2 and Ang(1-7) are expected to block the ACE/Ang II pathway and shift the RAS towards the ACE2/Ang(1-7) pathway to mitigate kidney injury. RAS, renin-angiotensin system; AGT, angiotensinogen; Ang I, angiotensin I; ACE, angiotensin-converting enzyme; Ang II, angiotensin II; ACE2, angiotensin-converting enzyme 2; AT1R, Ang II type 1 receptor; MasR, Mas receptor; ACEI, ACE inhibitor; ARB, angiotensin receptor blocker; SARS-CoV, severe acute respiratory syndrome coronavirus; hrsACE2, human recombinant soluble ACE2. Figure was adapted from South *et al.* [9] and Sparks *et al.* [10].

might be related to multiple factors, including the number of cases, patient age, and the severity of the infection. Of note, SARS-CoV-2 RNA was detectable in urine sediments from COVID-19 patients [17] and the signal of SARS-CoV nucleoprotein immunostaining was localized to renal tubules of COVID-19 patients [36]. Thus, SARS-CoV-2 may directly infect the human kidney.

Current knowledge suggests several potential mechanisms are responsible for AKI in COVID-19 patients. On the one hand, several indefinite kidney injury factors may be the unspecific pathogenesis for AKI development in COVID-19 patients [31]. On the other hand, the cell-entry receptors of SARS-CoV-2, including ACE2 and transmembrane protease, serine 2 (TMPRSS2) [43], are co-expressed in proximal tubular epithelial cells [44] and lung type II pneumocytes [45]. Along this line, the direct entry of SARS-CoV-2 into cells in the kidney may directly cause AKI [29,31,46]. However, other studies have indicated no evidence to support the direct SARS-CoV-2 infection in the kidney in COVID-19 patients, suggesting that natural infection by SARS-CoV-2 is unlikely to cause AKI [33,35]. Second, cytokine storm syndrome [47,48] and heightened adaptive immune responses [29,31,35] may contribute to COVID-19-related AKI. Third, an imbalance between ACE2-Ang(1-7)-MasR and ACE-Ang II-AT1R axis might contribute to COVID-19-related AKI. To support this notion, the binding of SARS-CoV-2 and ACE2 diminishes membrane-bound ACE2 abundance and promotes an Ang II accumulation to activate ACE-Ang II-AT1R signaling but block ACE2-Ang(1-7)-MasR axis, resulting in inflammation and fibrosis [31,49].

Fundamental Role of Endothelial Dysfunction in COVID-19

Endothelial dysfunction has been known as a common denominator of some diseases that increase the risk for severe COVID-19, including hypertension, diabetes, thrombosis, and kidney failure [50,51]. Indeed, increasing evidence suggests the relations between endothelial cells and SARS-CoV-2 infections, including the expression of the cell-entry receptors of SARS-CoV-2 in the vascular endothelial cells (ECs), the prevalence of endotheliitis in patients with COVID-19, and the evidence of EC infection with SARS-CoV-2 in patients with fatal COVID-19 [52–57]. Of note, ACE2 is essential for EC infection with SARS-CoV-2, as evidenced by the observation that SARS-CoV-2 was incapable of directly infecting primary human endothelial cells lacking ACE2 receptors. In contrast, ACE2 overexpression in these cells resulted in high viral titers and inflammatory responses during SARS-CoV-2 infection [58]. Importantly, endothelial injury and dysfunction may be both a cause and/or a consequence of severe COVID-19 that is directly caused by SARS-CoV-2 infection and indirectly as a result of the profound systemic inflammatory cytokine storm, thus may contribute to end-organ damage and thrombotic events in patients with severe COVID-19 [50,59–61]. Indeed, anticoagulants [62] and fibrinolytic drugs [63], targeting ECs, have been used and improved the outcomes of COVID-19 patients. Thus, preventing and improving endothelial dysfunction may be a good direction for COVID-19 therapy.

Imbalance Between ACE2-Ang(1-7)-MasR and ACE-Ang II-AT1R Axis Contributes to Kidney Injury

Pathogenic actions of intrarenal ACE-Ang II-AT1R axis on kidney injury

Increasing evidence has shown an essential role of the intrarenal ACE-Ang II-AT1R axis in renal disease progression. An unsuitable activation of intrarenal ACE-Ang II-AT1R signaling has been observed in various animal models of renal diseases, such as diabetic nephropathy [64,65], glomerulonephritis [66], hypertensive nephropathy [67], 5/6 nephrectomy [68], renal ischemia/reperfusion [69], and polycystic kidney disease [70]. RAS inhibitors, including AGT oligonucleotide [71], direct renin inhibitor aliskiren [71,72], ACEIs [73,74], and ARBs [75–77], protect against kidney injury and inhibit disease progression. In contrast, the intrarenal hemodynamic effect of ACEIs leads to time-dependent changes of circulating Ang II levels, and thereby the compromised renal function in the presence of a reduction in renal perfusion [78]. Circulating Ang II levels are acutely lowered by ACEI treatment, whereas its long-term treatment raises that back to the baseline levels [79]. These results indicate the dual effects of ACEIs on renal functions depending on the setting they are administered. Overall, the concept of the pathogenic actions of intrarenal ACE-Ang II-AT1R signaling in kidney injury has been well established.

Protective actions of intrarenal ACE2-Ang(1-7)-MasR axis on kidney injury

In contrast to the above discussed intrarenal ACE-Ang II-AT1R axis, the intrarenal ACE2-Ang(1-7)-MasR axis has been downregulated in multiple animal models of renal diseases, such as diabetic nephropathy [80–82], renal ischemia/reperfusion injury [69], subtotal nephrectomy [83], and experimental Alport syndrome [84], implying the potential protective function of intrarenal ACE2-Ang(1-7)-MasR signaling in response to experimental kidney injury. To date, a variety of studies have investigated the function of the ACE2-Ang(1-7)-MasR axis on experimental kidney injury employing both pharmacological and genetic approaches. Deletion of the *Ace2* gene or pharmacologic inhibition of ACE2 worsens renal injury accompanied by increased renal ACE expression and intrarenal Ang II level in multiple disease models [81,85–92]. In contrast, ACE2 overexpression or human recombinant soluble ACE2 (hrsACE2) protein infusion ameliorates diabetic kidney injury [74,93], Ang II-induced tubulointerstitial fibrosis [87], experimental Alport syndrome [94], and atherosclerotic renal injury [92,95], accompanied by diminished intrarenal Ang II level and augmented intrarenal Ang(1-7) level. Similarly, in a severe COVID-19 patient, intravenous delivery of hrsACE2 markedly reduced plasma Ang II level. However, it increased Ang(1-7) and Ang(1-9), resulting in a marked reduction in the inflammatory cytokines IL-6 and IL-8, with a significant clinical improvement of the treated patient [96].

Regarding the effect of Ang(1-7) on kidney injury, Shao *et al.* [97] reported that chronic exogenous Ang(1-7) administration through vein injection did not improve streptozotocin-induced diabetic rat renal injury but accelerated diabetic nephropathy progression. They found that Ang(1-7) injection increased ACE and AT1R expression but dramatically reduced AT2R, ACE2, and MasR expression in the kidney of diabetic rats [97]. However, several other studies have shown the renoprotective effects of Ang(1-7) in high-fat diet-fed mice [98], experimental Alport syndrome [99], and unilateral ureteral obstruction rats [100], in which renal AT1R expression was suppressed and renal ACE2 expression was enhanced. The reason for the discrepancy is unclear, but it could be associated with the distinctions in different experimental models. Therefore, these results consistently suggest the protective actions of intrarenal ACE2-

Ang(1-7)-MasR signaling on kidney injury, opening a promising avenue for the therapeutic potential of this non-classical RAS for renal disease.

Inconsistent effects of common RAS inhibitors on renal ACE2 expression/activity

Although the structure of ACE2 shares 40% homology and 61% similarity with that of ACE, ACE2 only acts as a homologous enzyme but not an isoenzyme for ACE, and the specificity of a substrate and the enzyme activity of ACE2 are entirely different from that of ACE [101]. Until now, the published data about the effect of RAS inhibitors on plasma ACE2 activity and Ang(1-7) level in non-COVID patients are still controversial [49], and multiple common RAS inhibitors exhibit conflicting effects on renal ACE2 expression/activity in different experimental models (Table 1). These inconsistent effects of RAS inhibitors on renal ACE2 may be associated with the distinctions in various experimental models and the specificity of the drugs. What is more, Ang II (400 ng/kg/min) and Ang II combined with losartan had no effect on renal ACE2 expression/activity in mice, which is not associated with the alteration of blood pressure [111]. Consistently, renal ACE2 expression in humans is not associated with hypertension and RAS inhibitors but positively correlated with the biochemical index of kidney function [106], indicating a renoprotective action of ACE2 in the absence of COVID-19.

Modulation of Renin-Angiotensin System in COVID-19 Patients

The data published so far on plasma RAS activity and ACE2 activity in patients with COVID-19 is controversial. Although it has been reported that there is no difference in the serum concentrations of ACE2, Ang II, and aldosterone between COVID-19 and regular patients [112,113], several other studies have reported the regulation of plasma RAS by COVID-19. Firstly, an *in vitro* study by Lu and Sun [114] has shown that SARS-CoV-2 trimeric spike protein enhances ACE2 proteolytic activity. Secondly, SARS-CoV infection significantly increased the transcription level of the *Ace2* gene in human bronchial epithelial cells [115]. Lastly, dramatic rises in the serum concentrations of Ang II, Ang(1-7), ACE2, and serum ACE2 activity were observed in severe COVID-19 patients [116–118]. High-affinity binding of SARS-CoV-2 spike protein increased ACE2-enzymatic activity [114]. Thus the enhanced serum ACE2 activity in these patients may be secondary to SARS-CoV-2 infection and reflect a compensatory pathophysiological mechanism that counterbalances an excess in Ang II.

What's more, it has been reported that interferons or viruses only induce the expression of a truncated ACE2 isoform (△ACE2) but not the full-length ACE2 [45,119]. The △ACE2 does not have ACE2-enzymatic activity and the ability to bind with the SARS-CoV-2 [119]. Thus, there is still no evidence for the upregulation of the natural ACE2 expression. On the other hand, a recent clinical study

Table 1. Effects of RAS inhibitors on ACE2 expression/activity in the kidney

RAS inhibitor/ Gene deletion	Target	Experimental models/patients	Renal ACE2 expression / activity	Ref.
Agt ASO	AGT	C57BL/6J mice (unknown age)	ACE2 expression ↔	[102]
Aliskiren	Renin	Streptozotocin-induced diabetic nephropathy rats	ACE2 expression ↓	[103]
Captopril	ACE	Old C57BLKS/J mice (12–14 weeks)	Total ACE2 expression ↔ Membrane ACE2 expression ↓ Cytosolic ACE2 expression ↑	[104]
Enalapril	ACE	C57BL/6J mice (unknown age)	ACE2 expression ↔	[102]
Ramipril	ACE	Subtotal nephrectomy rats	ACE2 activity ↑	[83]
Ramipril	ACE	Healthy young C57BL/6N mice (7–8 weeks)	ACE2 expression ↔	[105]
Ramipril	ACE	Aged C57BL/6N mice with diabetes (28 weeks)	ACE2 expression ↔	[105]
Perindopril	ACE	Spontaneously hypertensive rat	ACE2 expression ↔	[106]
Losartan	AT1R	C57BL/6J mice (unknown age)	ACE2 expression ↔	[102]
Losartan	AT1R	Spontaneously hypertensive rat	ACE2 expression ↔	[106]
Telmisartan	AT1R	Old C57BLKS/J mice (12–14 weeks)	Total ACE2 expression ↔ Membrane ACE2 expression ↓ Cytosolic ACE2 expression ↑	[104]
Telmisartan	AT1R	Healthy young C57BL/6N mice (7–8 weeks)	ACE2 expression ↔	[105]
Telmisartan	AT1R	Aged C57BL/6N mice with diabetes (28 weeks)	ACE2 expression ↔	[105]
Valsartan	AT1R	TG(mRen2)27 (Ren2) transgenic rats	ACE2 expression ↑	[107]
ACE.4	ACE	Mice with ACE kidney ablation	ACE2 expression/activity ↓	[104]
ACE8/8	ACE	Mice with ACE kidney ablation	ACE2 expression ↓ ACE2 activity ↔	[104]
ACEI/ARB	ACE/AT1R	Patients with diabetic kidney disease	ACE2 expression ↔	[16,108]
ACEI/ARB	ACE/AT1R	Hypertensive patients	ACE2 expression ↔	[106]

RAS, renin-angiotensin system; AGT, angiotensinogen; ASO, antisense oligonucleotides; ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme 2; AT1R, Ang II type 1 receptor; ACEI, ACE inhibitor; ARB, angiotensin receptor blocker; ↑, Up-regulation; ↓, Down-regulation; ↔, Unaffected. ACE.4 mice have the somatic ACE promoter replaced by the kidney androgen-regulated protein promoter, the levels of kidney ACE are less than 1% of normal and no ACE is detected in other organs [109]. ACE8/8 mouse is a model lacking ACE in the kidney or vascular endothelium, but with 100-fold normal cardiac ACE levels [110].

by Kutz *et al.* [120] showed that the serum levels of RAS peptides, including Ang I, Ang II, Ang(1-5), and Ang(1-7), as well as plasma renin activity in COVID-19 patients, were significantly decreased compared with non-COVID-19 patients. Yet, there is no difference in plasma ACE and ACE2 activity between the two groups [120]. The reasons for the discrepancy are unclear but could be related to the severity of the virus infection and the differences in detection methods. Notably, plasma Ang II/Ang I ratio was significantly suppressed in COVID-19 patients with ACEI treatment, which may contribute to increased plasma ACE2 activity [113].

Interestingly, both plasma renin and aldosterone concentrations [24] and the level of renal ACE2 [16,36] were found to be enhanced in COVID-19 patients with AKI compared to patients with no AKI. The enhanced plasma renin and aldosterone concentrations were strongly associated with AKI in COVID-19 patients [24], indicating a potential impact of RAS inhibitors on COVID-19 outcomes. Simultaneously, the enhanced renal ACE2 expression may establish a vicious circle of viral infection via the SARS-CoV-2/ACE2 complex endocytosis and trigger a compensatory response against AKI by activating the intrarenal ACE2-Ang(1-7)-MasR signaling. Thus, the enhanced ACE2 may play as a double-edged sword in the setting of COVID-19 disease. However, the level of classic RAS in the kidney in COVID-19 patients with AKI is still unknown. Based on the pathogenic actions of the classic RAS on kidney injury, we speculate that the classic RAS in the kidney is also activated and contributes to the progression of COVID-19-related AKI.

The Use of Soluble ACE2 in COVID-19

Either TMPRSS2-enhanced endocytosis of the SARS-CoV-2/ACE2 complex or ADAM17-induced ACE2 cell surface shedding [121] caused the downregulation of membrane-bound ACE2 level. It may be closely related to multi-organ dysfunction, including severe acute lung injury and AKI [3]. ACE2 is a functional receptor for SARS-CoV-2 infection [47], implying that competing for the binding of SARS-CoV-2 and membrane ACE2 to inhibit SARS-CoV-2 infections, especially the use of soluble ACE2 as a decoy for SARS-CoV-2, might be a potential strategy for the therapy of patients with COVID-19 [122]. In support of this notion, a series of hACE2 variants [123–126] and ACE2-derived peptides [127] were reported to successively neutralize SARS-CoV-2, thus blocking its infection potently. What's more, hrsACE2 was reported to successfully inhibit SARS-CoV-2 infection [128] and treat a severe COVID-19 patient [96]. hrsACE2 treatment did not interfere with the generation of neutralizing antibodies but caused the disappearance of the virus rapidly from the serum, decreased inflammatory cytokine levels, and blocked the systemic spread of the virus from the lung to other organs [96]. As an extension of this observation, Tada *et al.* [129] developed an improved soluble ACE2 by fusing Fc domain 3 of the immunoglobulin heavy chain to the ACE2 ectodomain with an $H^{345}A$ mutation. This ACE2 microbody lost the enzyme activity but exhibited a 10-time higher potency to inhibit virus infection and replication than soluble ACE2 [129]. Recently, Larue *et al.* [127] designed a panel of ACE2-derived peptides based on the binding of ACE2 and SARS-CoV-2 and observed efficient inhibition on virus replication and SARS-CoV spike protein-mediated virus infection. Therefore, drugs including but not limited to soluble ACE2 variants, ACE2 analogs/peptides, and ACE2 inhibitors may be the options in the acute management of COVID-19 by competing for binding to SARS-CoV-2 on the cell surface, but enzymatically active ACE2 variants may be the best options for improving outcomes of COVID-19 patients, since they may serve dual functions of competitive binding to SARS-CoV-2 and activation of the ACE2-Ang(1-7)-MasR signaling [130]. However, it still lacks direct evidence from the large-scale clinical trials. Further studies, especially large-scale clinical trials, are needed to evaluate the therapeutic value of the above proteins/peptides on the therapy of COVID-19.

However, several other receptors (**Table 2**) have already been reported for SARS-CoV-2 infections in the cell surface. In particular, TMPRSS2 inhibitor camostat mesylate significantly inhibited SARS-CoV-2 entry [43], suggesting that the infection of SARS-CoV-2 not only relies on ACE2 but also needs TMPRSS2. This result is in line with the findings reported by Heurich *et al.* [121] that TMPRSS2 is essential for direct membrane fusion of SARS-CoV by processing the cleavage of SARS-CoV spike protein. However, TMPRSS2 competes with ADAM17 for the cleavage of ACE2, but only the cleavage of ACE2 by TMPRSS2 augmented ACE2-bound viral endocytosis depends on Cathepsin L [121]. Therefore, ACE2 may not be the central factor for SARS-CoV-2 infections. Other proteins that mediate endocytosis may also contribute to SARS-CoV-2 virus entry, but ACE2 is important for SARS-CoV-2 infections, since hrsACE2 has been reported to successfully inhibit SARS-CoV-2 infection of organoids and Vero cells [44,128]. It is well-known that the incidence and severity of SARS-CoV-2 infection may be related to the ACE2 [139], but the incidence of serious infections is relatively higher in patients with multiple underlying diseases including diabetes, hypertension, and cardiovascular disease [26,140].

Table 2. Receptors/proteins for SARS-CoV-2 infection

Target	Full name	Ref.
ACE2	Angiotensin-converting enzyme 2	[43]
AGTR2	Angiotensin II receptor type 2	[131]
CTSL	Cathepsin L	[43]
CD147	Basigin or EMMPRIN	[132,133]
CD209L	CLEC4M and L-SIGN	[134]
CD209	DC-SIGN	[134]
KIM-1/TIM-1	Kidney injury molecule-1/T cell immunoglobulin mucin domain 1	[135]
NRP1	Neuropilin-1	[136]
OR	Olfactory receptor	[137]
RAGER	The receptor for advanced glycation end products	[138]
TMPRSS2	Transmembrane protease serine 2	[43]

Do RAS Inhibitors Directly Correlate with COVID-19?

Although several studies have shown the inconsistent effects of different RAS inhibitors on ACE2 expression/activity, it is unclear whether ACEIs/ARBs exhibit a harmful or beneficial effect on SARS-CoV-2 infection and COVID-19 outcomes in patients, which may depend on the severity of the cases and the type of complications. The data published so far on the impact of ACEI/ARB on clinical features of patients with COVID-19 is complicated. Many reports by meta-analysis have shown a protective effect and the safety of ACEI/ARB in COVID-19 patients [5,6,141–143]. Along this line, several retrospective cohort studies have obtained a similar conclusion on the effect of ACEI/ARB in COVID-19 patients [144–149]. The beneficial effect may be attributed to the suppressed Ang II/Ang I ratio in the plasma and increased plasma ACE2 activity in COVID-19 patients [113]; this needs to be clarified in future studies. However, a retrospective cohort study in Korea by Lee *et al.* [150] challenged that the use of RAS blockers was associated with a higher risk of SARS-CoV-2 infection in patients with hypertension. Similarly, Chan *et al.* [151] showed that ARB augmented the risk of SARS-CoV-2 infection in younger subjects without apparent effects on COVID-19 outcomes [151]. These reports implied that RAS inhibitors might aggravate COVID-19 by facilitating SARS-CoV-2 entry. The possible mechanism may involve the suppressed ACE2 endocytosis and ubiquitin-mediated degradation during AT1R inhibition [152]. Other reports also challenged that ARB/ACEI did not affect the risk of contracting COVID-19 [153,154]. What's more, chronic (long-term) treatment with ACEI/ARB increased the risk of AKI in severe COVID-19 patients, as reflected by the increased urea nitrogen [155]. Thus, the application of RAS inhibitors should be recommended with a comprehensive analysis of the underlying diseases in COVID-19 patients.

Conclusions

Kidney with high expression of ACE2 is one of the extrapulmonary target organs affected in patients with COVID-19, and COVID-19-related AKI is one of the independent risk factors for the death of COVID-19 patients. ACE2 is recognized as one of the pivotal factors for SARS-CoV-2 infections and an essential component of the ACE2-Ang(1-7)-MasR axis that might exhibit a protective effect on COVID-19-induced AKI. Imbalanced intrarenal RAS may contribute to COVID-19-associated AKI, derived from the theoretical derivation of intrarenal RAS. Further studies with multidisciplinary collaboration are needed to investigate the exact molecular mechanism of COVID-19-associated AKI. Although series of research have shown the inconsistent effects of multiple common RAS inhibitors on ACE2 expression and enzyme activity, most of the retrospective cohort studies indicated the safety and protective effects of ACEI/ARB in COVID-19 patients. Evidence-based RAS inhibitors cannot be arbitrarily discontinued in the current ambiguous situation, but the underlying diseases in COVID-19 patients should be carefully considered. Until now, there is no specific drug for COVID-19 treatment. Drugs such as hrsACE2 and ACE2 variants with preserved ACE2-enzymatic activity not only compete for the binding of SARS-CoV-2 and membrane ACE2 to inhibit SARS-CoV-2 infection but also activate the ACE2-Ang(1-7)-MasR signaling to protect against tissue/organ injury; these may be the best options for improving outcomes of COVID-19 patients associated with AKI. For instance, an enzymatically active ACE2 variant fused with a 5-kD a albumin-binding domain (ABD) and bridged via a dimerization motif hinge-like 4-cysteine dodecapeptide (ACE2 1-618-DDC-ABD) exhibited prolonged duration of plasma ACE2-enzymatic activity and 20- to 30-fold higher binding affinity to SARS-CoV-2 and protected against lethal disease caused by SARS-CoV-2 infection in transgenic k18-hACE2 mice [156]. Overall, ACE2 1-618-DDC-ABD may be a clinically applicable therapeutic candidate for COVID-19, and future clinical studies are needed to evaluate its therapeutic value in patients with COVID-19.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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