

Rebalance of the RAAS axes: the therapeutic strategy of COVID-19?

Wenhui Huang¹, Ying Meng¹, Pingsheng Wu¹, and Xu Li¹

¹Southern Medical University Nanfang Hospital

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Abstract

COVID-19 has been the biggest global health threat since world war II. Up to now, the novel coronavirus pneumonia epidemic has resulted in over 300 thousand deaths. Angiotensin-converting enzyme 2 (ACE2), the receptor of SARS-CoV-2, is also the key modulator of renin-angiotensin-aldosterone system (RAAS) axes, which regulates the inflammatory response to maintain internal environment balance. On the contrary, RAAS axes can also regulate the expression of ACE2 which may influence on the susceptibility of SARS-CoV-2. Furthermore, SARS-CoV-2 binds with ACE2 and subsequently down-regulates ACE2 expression, which may exacerbate the inflammatory response due to RAAS imbalance. In this review, we summarized pathogenesis and clinical characteristics of COVID-19 patients, and discussed the potential link between the RAAS axes imbalance and the susceptibility/progression of organ injury in COVID-19. Therefore, we speculate that rebalance of the RAAS axes is the preventive and therapeutic strategy of COVID-19.

Abbreviations list:

ACE Angiotensin converting enzyme
ACE2 Angiotensin converting enzyme-2
RAAS Renin-angiotensin-aldosterone system
Ang I Angiotensin I
Ang II Angiotensin II
AT1R Ang II receptor type 1 receptor
AT2R Ang II receptor type 2 receptor
Ang (1-7) Angiotensin (1-7)
MasR Mas receptor
MrgD MAS-related G protein-coupled receptor D
ACEI Angiotensin converting enzyme inhibitor
ARB Angiotensin receptor blocker
rhACE2 recombinant human ACE2
ADAM17 disintegrin and metalloprotease 17
TMPRSS2 transmembrane protease serine 2
SARS severe acute respiratory syndrome

ALI Acute lung injury

ARDS Acute respiratory distress syndrome

CNS Central nervous system

COVID-19 Coronavirus disease-19

IBD Inflammatory bowel disease

LPS lipopolysaccharide

eNOS endothelial nitric oxide synthase

1. Introduction

Since the start of the latest coronavirus (SARS-CoV-2) outbreak, the number of asymptomatic infected individuals and cases of coronavirus disease (COVID-19) have been increasing exponentially worldwide, which has been the biggest global health threat after world war II. COVID-19 is like to severe acute respiratory syndrome and mainly affects the respiratory tract and can subsequently result in acute respiratory distress syndrome (ARDS) and multi-organ failure(Huang *et al.* , 2020). Studies have proved that SARS-CoV-2, like SARS-CoV, is a family member of beta coronaviruses(Wang *et al.* , 2020) and 96% identical at the whole-genome level to a bat coronavirus(Zhou *et al.* , 2020). Meanwhile, the SARS-CoV-2 virus has been reported that it uses angiotensin converting enzyme-2 (ACE2) as a cellular entry receptor via the spike protein (S). The interesting thing is that SARS-CoV-2 binds ACE2 with higher affinity than SARS-CoV(Wrapp *et al.* , 2020), which meant that it is difficult to control. ACE2 is a key modulator of the renin-angiotensin-aldosterone system (RAAS), which is associated with blood pressure homeostasis, oxidative stress, vasoconstriction, fibrosis and inflammation(Turner *et al.* , 2002). The infection of SARS-CoV-2 may decrease the ACE2 expression and subsequently induce the inflammation in various organs. Moreover, accumulating evidence has demonstrated that ACE2 may be essential in the progression and clinical outcomes of COVID-19(Xu *et al.* , 2020). Therefore, we will review the role of ACE2 in various conditions, and discuss its potential implication in the susceptibility and progression of COVID-19.

2. The infected organs and transmission in COVID-19 patients

Pathology and clinical symptoms indicated that SARS-CoV-2 can do harm to multiple organs. Therefore, it is important to understand the possible infected organs and route of transmission of SARS-CoV-2. Report showed that SARS-CoV-2 has the higher aerosol and surface stability than SARS-CoV(van Doremalen *et al.* , 2020). The human-to-human viral transitions of the SARS-CoV-2, reported by the previous studies, occurs due to close contact with an infected person, exposed to coughing, sneezing, respiratory droplets or aerosols. These aerosols can penetrate the human body (lungs) via inhalation through the nose or mouth. Therefore, the World Health Organization (WHO) has suggested that the personal protective equipment and infection control guidelines should be based on the assumption that the primary mechanism of transmission is direct and indirect droplet spread.

Direct droplet spread is said to occur when respiratory particles greater than 5 μ m in diameter make contact with the mucosal surface of a recipient(Wilson *et al.* , 2020). Direct droplets can enter the human respiratory system through air(Jiang *et al.* , 2020), which causes respiratory symptoms. COVID-19 patients with respiratory distress could produce high levels of aerosols secondary to cough, high airway pressures, minute volumes, altered secretions and basal collapse. When infected patients expel respiratory particles and someone would inhale them, and subsequently the SARS-CoV-2 would find a welcome home in the nose(Sungnak *et al.* , 2020). High-flow nasal oxygen would disperse a concentrated jet of aerosols, potentially spreading them over a further distance or into the alveoli. These particles could gain direct access to alveolar surface ACE2 receptors under suitable biological, physical and environmental conditions. If the immune system does not beat back SARS-CoV-2 during this initial phase, the virus then marches down the windpipe to attack the lungs, where it can turn deadly. Several reports have reported that occur within families(Chan *et al.* , 2020). After the SARS-CoV-2 enters into the blood, it would invade different organs by binding to ACE2

receptor. Although the lungs are ground zero, the SARS-CoV-2 would reach to many organs including the heart, kidneys, gut, brain, blood vessels and reproductive system. Clinical trials have demonstrated that the infected patients not only have respiratory symptoms, but have kidney, brain, reproductive system, gut and liver injury. SARS-CoV-2 may enter different organs through the hematogenous transmission. Coronavirus invades the central nervous system (CNS) through the olfactory bulb(Bohmwald *et al.* , 2018). The retrospective, observational case series have reported the COVID-19 patients may appear neurologic manifestations(Chen *et al.* , 2020; Mao *et al.* , 2020), including central nervous system manifestations (dizziness, headache, seizure and so on), and peripheral nervous system manifestations (nerve pain, taste impairment, smell impairment and so on). Although the clinical manifestation of COVID-19 is dominated by respiratory symptoms, some patients have severe cardiovascular damage(Huang *et al.* , 2020; Wang *et al.* , 2020), which may be related to the distribution of ACE2 in heart tissue. At the same time, acute kidney injury could also be induced by the virus(Guan *et al.* , 2020; Huang *et al.* , 2020; Wang *et al.* , 2020), which may be related to cytokine storm resulting from respiratory symptoms. Moreover, the liver injury should not be ignored because abnormal liver enzymes in serum was found in the hospitalized patients(Liu *et al.* , 2020; Weber *et al.* , 2020; Xu *et al.* , 2020). Furthermore, the glycemic variability was associated with composite adverse outcomes and death in COVID-19 patients(Zhu *et al.* , 2020). Moreover, impairment of reproductive health induced by SARS-CoV-2 may be caused by high levels of cytokines resulted from respiratory symptoms(Wang *et al.* , 2020). These complications in various organ mainly demonstrated the transmission of virus in blood.

The respiratory droplets transmission is the main route of infection, while there is indirect droplet spread, including contact transmission and Fecal-Oral transmission. Although there is little reported about the contact transmission, it still an important way in infection. The contact transmission is mainly touched by a hand, which then contacts mucosal surfaces. The SARS-CoV-2 patients have found the presence of virus in tears and conjunctival secretions(Xia *et al.* , 2020), while a single-center cross-sectional study found the risk of infection on ocular surface is low(Zhang *et al.* , 2020). Meanwhile, the SARS-CoV-2 could be found in the nasal cavity. The Fecal-Oral transmission is also possible in COVID-19, as virus was noted in stools(Lin *et al.* , 2020; Tang *et al.* , 2020; Zhang *et al.* , 2020). At the same time, some patients appeared gastrointestinal symptoms including diarrhea, vomiting and abdominal pain during course of the disease(Jin *et al.* , 2020; Young *et al.* , 2020). This may be related to the virus that could be detected in Oral or saliva, subsequently infected along the digestive tract(Azzi *et al.* , 2020). Altogether, the transmission and the infected organs were displayed in **Figure 1** and **Table 1** .

3. ACE2 is the modulator of RAAS

Angiotensin-converting enzyme 2 (ACE2), the main active peptide of renin-angiotensin-aldosterone system (RAAS), is a homologue to the carboxypeptidase angiotensin-converting enzyme (ACE), which generates angiotensin II (Ang II). As the name of RAAS indicates, renin and angiotensin are two critical components forming the system. Renin is the first and rate-limiting step in the RAAS cascade, without the renin enzyme there are no angiotensin. Angiotensinogen is the precursor to all angiotensin and is converted via renin to form angiotensin I (Ang I). Under the physiological conditions, the protease renin cleaves angiotensinogen to generate Ang I, then ACE cleaves Ang I to Ang II, which contact with Ang II receptor type 1 receptor (AT1R) and Ang II receptor type 2 receptor (AT2R). The proinflammatory effects of Ang II are mediated through AT1R(Fleming *et al.* , 2006). Studies have shown that the ACE/ Ang II/ AT1R axis can induce vasoconstriction, hypertension, inflammation, proliferation and fibrosis, and ACE is the key enzyme in the regulation of Ang II production. However, in 2000, the homologue of ACE, ACE2 has been discovered that it negatively regulates the activated renin-angiotensin system by degrading Ang II to the heptapeptide angiotensin (1-7) (Ang (1-7)), which reacts with MAS receptor (MasR)(Rice *et al.* , 2004). There are various methods to regulate the balance between ACE/ Ang II/ AT1R axis and ACE2/Ang (1-7)/Mas, including recombinant human ACE2 (rhACE2), ACE inhibitors (ACEI) and angiotensin receptor blockers (ARBs). As reported, ACE2 has a strong affinity with Ang II type 1 and type 2 receptors, thus it has been identified as an important counter regulatory mechanism to the classic RAAS. ACE2 controls the pressor/depressor balance of the RAAS by i) converting angiotensin I to angiotensin (1-9), limiting the number of substrate

available to generate angiotensin II, ii) converting angiotensin II to angiotensin (1-7), restricting angiotensin II stimulation of the AT1 receptor and iii) generation of angiotensin (1-7), which is capable of binding to its own receptor, Mas receptor to oppose the pressor actions of the AT1 receptor. The ACE2/Ang (1-7)/Mas receptor axis has been shown to play protective roles in numerous disease models including heart failure, hypertension, injury, fibrosis and metabolic syndrome(Mirabito *et al.* , 2019). Upregulating ACE2 may transfer the balance of RAAS to the ACE2/Ang (1-7)/Mas axis for protection. Most available evidence supports a counter-regulatory role for Ang (1-7) by opposing many actions of Ang II on AT1R receptors, especially vasoconstriction, inflammation and proliferation. In addition, ACE2 also has been confirmed as an essential receptor for SARS coronavirus infections as well as a protective molecule against lethal lung failure in SARS(Liet *et al.* , 2003). In fact, SARS receptor function of ACE2 is independent of its catalytic activities for Ang II degradation, whereas ACE2-mediated Ang II degradation is important for lung protection from SARS pathogenesis.

4. ACE2 is expressed in various organs and plays an important role in organ injury secondary to COVID-19

According to the latest clinical research, the infected patients not only have respiratory symptoms, but also have cardiovascular, digestive, central system, renal and other symptoms(Guan *et al.* , 2020). ACE2 is a type I integral membrane glycoprotein widely expressed in lung, heart, endothelium, kidneys, gastrointestinal tract and so on(Zou *et al.* , 2020). Based on the findings, the organs, which highly express ACE2, may be vulnerable to SARS-CoV-2 infection. The decrease of ACE2 expression may accelerate lung injury because ACE2 is a protective molecule of RAAS, which induce the inflammatory response and organ damage. Binding to the ACE2 receptor is a critical initial step for SARS-CoV-2 to entry into target cells. In the process of infection, it is likely to require two kinds of transmembrane proteases: ADAM17 metalloproteinase and TMPRSS2 transmembrane protease. ACE2 could be cut by ADAM17 metalloproteinase, which hinders the binding of virus and ACE2(Lambert *et al.* , 2005); while TMPRSS2 transmembrane protease is required to the process, assisting the combination of ACE2 and SARS-CoV-2(Iwata-Yoshikawa *et al.* , 2019). SARS-CoV-2 appears not only to gain the initial entry through ACE2 but also subsequently to down-regulate ACE2 expression, which leads to the immune response imbalance caused by ACE/Ang II/AT1R activation(Vaduganathan *et al.* , 2020). The down-regulation of ACE2 reduces the level of Ang (1-7), and subsequently increases the production of Ang II, which activates the RAAS and enhances inflammation. (**Figure 2**)

4.1 The respiratory system damaged in COVID-19

The lung is the most preliminary target organ for SARS-Cov-2 infection, and pneumonia is the most common complication seen in COVID-19 patients with the occurrence of 79.1%(Xie *et al.* , 2020). As reported, ARDS is one of the most severe complications in COVID-19 patients(Wang *et al.* , 2020). The down-regulation of ACE2 plays an important role in the pathogenesis of severe lung failure after SARS-CoV infection(Kuba *et al.* , 2005). Recent data showed that COVID-19 patients with COPD as coexisting disorder were more likely to progress into severe cases, which may be related with the decrease of ACE2. It has been reported that ACE2 exerted protective effects against multiple organs injury via degrading Ang II to Ang (1-7)(Kuba *et al.* , 2010). For example, ACE2 knockout mice showed severe ARDS-like injury pathology in the lungs comparing to the wild type controls. At the same time, the respiratory symptoms can be alleviated by the treatment of rhACE2 protein(Imai *et al.* , 2005). Meanwhile, a phase II clinical study found that the level of Ang II decreased rapidly in serum and Ang(1-7) increased after patients with ARDS treated with rhACE2(Khan *et al.* , 2017). Administration of rhACE2 could ameliorate H5N1-induced lung injury in mice(Zou *et al.* , 2014). Furthermore, previous studies of ACE insertion/deletion (I/D) polymorphism correlation with severity of ARDS in humans(Jerng *et al.* , 2006; Marshall *et al.* , 2002) and ACE inhibitors treatments in rodent ARDS(Raiden *et al.* , 2002) have suggested that the RAAS could have role in ARDS/acute lung injury (ALI). In addition to the treatment of rhACE2 and ACEI/ARB, Ang (1-7) also has a positive effect on the process. The ratio of ACE/ACE2 was positively correlated with the ARDS and Ang (1-7) could prevent the symptoms due to the increase of ACE2(Wösten-van *et al.* , 2011). Infusions of Ang (1-7) prevented rats from acute lung lesions after oleic acid administration as seen through a decrease in lung edema, myeloperoxidase

activity, histological lung injury score, and pulmonary vascular resistance(Klein *et al.* , 2013). The same results could be observed in a pulmonary injury model based on lipopolysaccharide (LPS)(Chen *et al.* , 2013). These results convincingly proved that ACE2 is protective against lung injury and upregulation of the ACE2/Ang (1-7) axis may be a therapeutic target for patients with COVID-19.

4.2 The intestinal homeostasis disorder in COVID-19

It was reported that many COVID-19 patients appeared gastrointestinal symptoms, like diarrhea, abdominal pain and vomiting(Guan *et al.* , 2020; Holshue *et al.* , 2020; Jin *et al.* , 2020). SARS-CoV-2 RNA not only exists in lung, but also in anal swabs and stool specimen(Tang *et al.* , 2020; Zhang *et al.* , 2020). ACE2 was confirmed to be expressed in gastrointestinal epithelial cells by sequencing, and it was positively correlated with gene expression related to virus release(Zhang *et al.* , 2020). Consequently, the gut may be the target of SARS-CoV-2. While the mechanism of gastrointestinal symptoms is not fully understood, current data indicated these histological changes could be caused by the infection of SARS-CoV-2, especially the down-regulation of ACE2 in tissue. The down-regulation of ACE2 expression will lead to the imbalance of RAAS, which causing inflammation in digestive system. It was found that the level of serum Ang II increased in the infected patients(Liu *et al.* , 2020), who have obvious clinical manifestations, such as diarrhea and liver injury with raised enzymes. Nevertheless, no viral inclusion was observed in the liver. Many studies have shown that the RAAS is related with the gut inflammation and gut microbiota. Treatment with Ang II may alter selectively gut microbial communities, and induce pathological alterations in gut wall(Sharma *et al.* , 2019). Previous animal experiments have shown that the expression of ACE2 and Ang (1-7) play an essential role in the gastrointestinal inflammation. The study of Hashimoto has confirmed that transplantation of the altered microbiota from ACE2 mutant mice into germ-free wild-type hosts was able to transmit the increased propensity to develop severe colitis(Hashimoto *et al.* , 2012). ACE2 interacts with B0AT1 amino acid transporter, which is necessary for polarized surface expression of the transporter in intestinal epithelial cells, and subsequently reduces the levels of tryptophan and other large amino acids, which resulted in alterations in gut inflammation and gut microbiota. Furthermore, systemic ACE2 deficiency would synergize to adversely impact both the microbiome and gut barrier function(Duan *et al.* , 2019). Meanwhile, inhibition of ACE2 may reduce DSS-induced inflammatory bowel disease (IBD) of mouse(Chen *et al.* , 2020). Moreover, treatment of the ACEI is able to upregulate ACE2 expression in injured liver(Huang *et al.* , 2010). In clinical trials, the IBD patients together with ACEI/ARB exposure had fewer hospitalizations, operations and corticosteroid prescriptions compared to matched controls(Jacobs *et al.* , 2019). All the above evidences have demonstrated that activation of the ACE2/Ang (1-7)/Mas signaling pathway or inhibition of the ACE/Ang II/AT1R pathway may be important treatments for gastrointestinal inflammation. Therefore, the imbalance of RAAS caused by SARS-CoV-2 infection may has adverse effects on the gut inflammation and gut microbiota, and gut-liver axis, which may participate in the pathological process of COVID-19 patients with sepsis. We speculate that rebalance of RAAS restore the intestinal homeostasis.

4.3 Cardiovascular system impairment in COVID-19

Patients with COVID-19 may have higher risk to develop complications and progress into severe cases when having coronal heart disease(Huange *et al.* , 2020). Among the hospitalized patients infected SARS-CoV-2, acute cardiac injury is one of the common complications. The cardiac injury found in COVID-19 patients may be at least partially attributed to the fluctuation of ACE2 expression. Former researches have proved that elevated cardiac and plasma Ang II levels were correlated with the heart contractility impairment after deletion of ACE2(Gurley *et al.* , 2006; Yamamoto *et al.* , 2006). It was found that ACE2 has the potential possibility to remodel and regulate heart function because ACE2 knockout mice has severe cardiac dysfunction(Nakamura *et al.* , 2008). Knockout mice of ACE genes or treatment with AT1 receptor blockers reversed the cardiac phenotype in ACE2 knockout mice(Oudit *et al.* , 2007). Similarly, the age-dependent cardiomyopathy in ACE2 knockout mice is likely mediated by Ang II-induced oxidative stress and inflammation through AT1 receptor by PI3K signaling. Meanwhile, the correlation between the activity of ACE2 in serum and the deterioration of heart failure has been confirmed(Goulter *et al.* , 2004). Especially, in the development from hypertension to heart failure, the decrease of serum of ACE2 activity suggested

a selective biomarker of cardiac systolic dysfunction(Pan *et al.* , 2018). Further *in vivo* murine studies showed Ang II mediated loss of membrane-bound cardiomyocyte ACE2 correlated with the upregulation of TACE/ADAM 17 activity which was prevented by AT1 receptor blockade(Weber *et al.* , 1991). Cardiac fibroblasts and coronary endothelial cells also express ACE2 and TACE and this reciprocal relationship extends to these cell types as well(Mehta *et al.* , 2007). In addition to counterbalancing Ang II/AT1R axis, the involvement of Ang (1-7) in the action of ACE2 on regulating heart function has been demonstrated. It is reported that the ACE2/Ang (1-7)/MasR axis plays an importance role in the regulation of RAAS system(Patel *et al.* , 2016). Treatment with Ang (1-7) peptide has been shown to improve myocardial performance, cardiac remodeling, and even survival in rodent heart failure models(Abwainy *et al.* , 2016; Santos *et al.* , 2004). All these evidences have proved that the ACE2/Ang (1-7)/MasR axis plays a very important role in cardiovascular system. Consequently, the impairment of cardiac function in COVID-19 patients is related to the down-regulation of ACE2/Ang (1-7)/MasR axis, which implicates the rebalance of RAAS in the treatment of COVID-19 with cardiovascular system impairment.

4.4 Acute kidney injury in COVID-19

Acute kidney injury is one of the severe complications in COVID-19 patients(Huang *et al.* , 2020) because ACE2 is abundantly expressed in kidney and the activity of ACE2 is even higher in the renal cortex than renal medulla(Wysocki *et al.* , 2010). The prevalence of kidney impairment (hematuria, proteinuria and kidney dysfunction) in hospitalized COVID-19 patients was high(Cheng *et al.* , 2020). ACE2 highly expresses in renal tubular cells, Leydig cells and cells in seminiferous ducts by analyzing ACE2 expression on the online datasets(Fan *et al.* , 2020). The expression of ACE2 has been confirmed to be related with acute kidney injury and several models of chronic kidney disease induced by hypertension(Fang *et al.* , 2013; Soler *et al.* , 2013). ACE2 has emerged as a protective molecule against kidney diseases(Oudit *et al.* , 2009). ACE2 knockout can lead to blood pressure rise, glomerular injury and renal fibrosis in diabetic mice(Liu *et al.* , 2017). Meanwhile, deletion of ACE2 leads to late-onset nephrotic glomerulosclerosis spontaneously(Oudit *et al.* , 2006). On the contrary, the increase of Ang II in the damaged renal tubules may be a possible mediator for further renal damage in human renal diseases. Ferrario found that ACE2 in renal cortex activity was significantly augmented in rats medicated with ACEI/ARB but not changed in the group which given the combination(Ferrario *et al.* , 2005). Furthermore, the high plasma levels of Ang (1-7) also have effects in the kidney. Ang (1-7) administration attenuated kidney injury without affecting blood glucose levels in Akita mice(Shi *et al.* , 2015). In addition, Ang (1-7) could ameliorated the renal injury of nephrectomized rats, and the combination of Ang (1-7) treatment alongside losartan exerted a superior effect to that of Ang (1-7) alone on regression of glomerulosclerosis(Xu *et al.* , 2013). The physiological effects reported for alamandine, the novel RAAS mediator, were similar to Ang (1-7), including vasodilation, anti-fibrosis, anti-inflammation and so on. The higher alamandine in plasma was associated with the renal size and functions(Paquette *et al.* , 2018). At the same time, alamandine level in plasma of patients was fluctuating and was related with chronic renal failure(Hrenak *et al.* , 2016). In conclusion, clinicians should pay attention to the risk of testicular lesions in patients of COVID-19.

4.5 Central nervous system impairment in COVID-19

The symptoms of SARS-CoV-2 infection usually appeared in the human respiratory system, while coronavirus infection has been associated with neurological manifestations. Upon nasal infection, coronavirus invades the central nervous system (CNS) through the olfactory bulb, causing inflammation and demyelination(Bohmwald *et al.* , 2018). For example, a retrospective case series of 214 patients has been analyzed that 25% patients have CNS manifestation: headache (13%), dizziness (17%), impaired consciousness (8%) and so on, while they did not perform clearly electroencephalography (EEG) or cerebrospinal fluid (CSF) analysis(Mao *et al.* , 2020). Another retrospective study has confirmed the occurrence of CNS following COVID-19(Chen *et al.* , 2020). As a matter of fact, the evidence on the CNS involvement of COVID-19 is scarce but still worthy of more attentions, because the infection of SARS-CoV-2 may partially explain why some patients develop respiratory failure. The entry of SARS-CoV-2 into human host cells is mainly mediated by the cellular receptor ACE2, thus the CNS manifestation may be associated with the expression

of ACE2 in brain. In K18-hACE2 (hACE2 expression was driven by K18 promoter) mice, the brain was a major target organ for SARS infection(McCray *et al.* , 2007). The virus entered the brain primary via the olfactory bulb resulting in rapid, transneuronal spread to other areas of the brain and finally induced the death of experimental animal(Netland *et al.* , 2008). Although it is limited to explore the occurrence of CNS following COVID-19, there are lots of evidences that have proved the function of ACE2 in brain injury or stroke. Accordingly, ACE2-deficient mice showed increased oxidative stress in the brain and autonomic dysfunctions compared to controls(Xia *et al.* , 2011). However, in a stroke model triggered by middle cerebral artery occlusion ACE2 overexpression in the brain resulted in a decreased stroke volume and improved neurological scores in mice(Chen *et al.* , 2014; Zheng *et al.* , 2014). Moreover, the human RAAS transgenic mice showed enhanced cerebral damage in experimental ischemia models which again could be prevented by additional transgenic expression of ACE2 in neurons(Zheng *et al.* , 2014). Further experiments have demonstrated an intracellular expression of ACE2 based on immunohistochemistry in different areas of the brain(Doobay *et al.* , 2007) and Ang (1-7) expression in primary neuronal cell culture from hypothalamic-brain stem areas(Gironacci *et al.* , 2013). Interestingly, it has been reported that in this brain region Ang (1-7) can be formed independent of Ang II processing(Pereira *et al.* , 2013). Using MAS antagonists, the authors revealed that ACE2 exerts its beneficial effects in ischemic brain injury by shifting the balance between Ang II and Ang (1-7) in favor of the latter, thereby reducing local reactive oxygen species production(Regenhardt *et al.* , 2013). Numerous reports showed that ACE2/Ang (1-7)/Mas axis has beneficial effects in brain injury(Bennion *et al.* , 2015; Bennion *et al.* , 2018; Jiang *et al.* , 2012). At the same time, the potential cerebroprotective effects of Ang (1-7) were also examined in a model of haemorrhagic stroke produced by intra-striatal injection of collagenase in normotensive rats(Delet *et al.* , 1996). While neurological manifestations of COVID-19 have still explored, it is highly likely that some of these patients have the susceptibility due to the distribution of ACE2 on central nervous system.

4.6 Hyperglycemia in COVID-19

Although there is no related epidemiological study about the diabetes in COVID-19 patients, we speculate that the patients with diabetes may have worse prognosis. A retrospective study published in *LANCET* found that half of patients infected with COVID-19 were associated with chronic diseases(Chen *et al.* , 2020), mainly including diabetes, cardiovascular diseases and digestive system diseases. Meanwhile, another retrospective study has found that 64 patients (46.4%) have more than one chronic diseases, while 14 patients (10.15%) with diabetes(Wang *et al.* , 2020). A retrospective, multi-centered study of 7337 cases of COVID-19 in Hubei Province, China, showed that patients with type 2 diabetes required more medical interventions and had a significantly higher mortality (7.8% versus 2.7%) and multiple organ injury than the non-diabetic individuals(Zhu *et al.* , 2020). Furthermore, they also suggested well-controlled blood glucose (glycemic variability within 3.9 to 10.0 mmol/L) was associated with markedly lower mortality compared to individuals with poorly controlled blood glucose. In coincidence, the COVID-19 patients with diabetes occurred hyperglycemia frequently and have higher mortality than patients without diabetes among 1122 patients reported in the United States(Bode *et al.* , 2020). These evidences indicated diabetic patients are likely to develop into severe cases once infection of SARS-CoV-2 occurs. ACE2 modulated the expression of neutral amino acid transporter on the insulin secretion pancreatic β -cells and the growth of pancreatic islet cells(Kuba *et al.* , 2010). The diabetic mice model expressing human ACE2 increased in the production of insulin, while decreased in apoptosis of pancreatic islet(Lu *et al.* , 2014). Local and systemic Ang II levels were regulated by ACE2, which was reduced in diabetic tissues(Tikellis *et al.* , 2012). Moreover, the ratio of ACE/Ang II was positively correlated with the systolic pressure, the fasting blood glucose level, serum creatine in the patients(Mizuiriet *et al.* , 2008). However, ACE2-deficient mice showed impairments in islet dysfunction and abnormality in glucose tolerance(Bindom *et al.* , 2010). Moreover, Endogenous ACE2 seems to have an effect on the adaptive β -cells hyperinsulinemic response, as seen in a diet-induced diabetes type 2 model, in which β -cells mass and proliferation were significantly reduced in ACE2-/- mice compared with controls(Shoemaker *et al.* , 2015). Interestingly, treatment with rhACE2 prevented from diabetic nephropathy(Tikellis *et al.* , 2003). These studies suggested the relationship between ACE2 expression and hyperglycemia, while it still needed to explore the mechanism that COVID-19 patients are more prone

to hyperglycemia in the status of ACE2 down-regulation.

4.7 Urogenital injury in COVID-19

The *NATURE* has showed that the testosterone to luteinizing hormone (T to LH) ratio in 81 patients with COVID-19 was decreased in comparison with 100 age-matched healthy counterparts(Wang *et al.* , 2020). Serum T to LH ratio could be a potential marker of impairment of reproductive health by SARS-CoV-2. They considered that high levels of cytokines following viral or bacterial infection, illness or injury can cause deterioration in spermatogenesis and steroidogenesis, adversely affecting fertility. In the same time, the other study has demonstrated that females were able to achieve viral clearance significantly earlier than males, while they also have found that the ACE2 expression in testes, accompany with the lungs and kidneys, was highest consulting in three independent databases(Shastri *et al.* , 2020). Using online datasets, Fan and colleagues clearly demonstrated that ACE2 is highly expressed in Leydig cells and cells of the seminiferous tubules in the human testis(Fan *et al.* , 2020). Interestingly, very little expression of ACE2 was found in ovarian tissue. Instead of the SARS-CoV-2, viruses such as HIV, hepatitis B and C, mumps and papilloma have been proved that they can cause viral orchitis and even lead to male infertility and testicular tumors(Feichtinger *et al.* , 1992; Poretsky *et al.* , 1995; Tessler *et al.* , 1987). Meanwhile, the testis autopsy specimens obtained from six patients who died of SARS-CoV showed that the virus could induce orchitis(Xu *et al.* , 2006). Surprisingly, SARS-CoV-2 particles could be found in the epithelial cells of testicular seminiferous tubules and Leydig cells, providing direct evidence of viral testicular damage(Fan *et al.* , 2020). Evidences have identified that ACE2 was highly expressed in human testis(Donoghue *et al.* , 2000; Harmer *et al.* , 2002; Tipnis *et al.* , 2000), which suggested the testis has the potential possibility to be infected by the SARS-CoV-2 virus. The combination of SARS-CoV-2 with these ACE2-positive cells provided a site for infection and caused severe alteration of testicular tissue. Therefore, the urogenital organs should be a significant concern when evaluating the prognosis of COVID-19.

5. Therapeutic strategy of COVID-19 by rebalancing the RAAS axes

There are several potential approaches to address ACE2-mediated COVID-19. On the one hand, selectively blocking the combination of S protein and ACE2 may be an effective route; On the other hand, rebalancing the RAAS axes may improve inflammation.

5.1 Blocking the combination of S protein and ACE2

Alanine scanning mutagenesis analysis was performed to identify determinants on ACE2 critical for SARS-CoV infection. The researchers found that charged amino acids between residues 22 and 57 were important, K26 and D30, in particular(Han *et al.* , 2006). At the meanwhile, Matthew found that a novel ACE2 inhibitor, NAAE, is effective in blocking the SARS coronavirus spike protein-mediated cell fusion by a structure-based approach(Huentelman *et al.* , 2004). Based on the combination of S protein and ACE2 in COVID-19, the structures of full-length human ACE2 have been revealed for the first time, and the dimeric ACE2 can accommodate two S protein trimers in SARS-CoV-2(Yan *et al.* , 2020). After the discovery of structures of full-length human ACE2, one study has determined the key region in SARS-CoV-2 that is responsible for the interaction with human ACE2(Wang *et al.* , 2020). Moreover, Shang and his colleague confirmed that a four-residue motif (residues 482-485: Gly-Val-Glu-Gly) could change the interface of SARS-CoV-2 RBM and ACE2, which may provide a potential target for intervention strategies(Shang *et al.* , 2020). According to these discoveries, researchers determined the Mpro and RdRp were the mainly target of infection by analyzing the RNA polymerase(Gao *et al.* , 2020; Jin *et al.* , 2020). Although it is the most fundamental method to block the combination of ACE2 and SARS-CoV, this method is time-consuming and costly. Meanwhile, it is difficult to predict the variation of coronavirus.

5.2 Rebalancing the RAAS axes by rhACE2

SARS-CoV-2 infection reduced ACE2 activity and increased the receptor consumption, further exacerbating pathophysiological changes, such as ACE/ACE2 ratio imbalance. Accordingly, there are several potential approaches to address ACE2-mediated COVID-19, including increases the expression and the activity of ACE2

to regulate the imbalance of RAAS. The imbalance of RAAS may result in abnormal high blood pressure and further induce multiple organ damage. Activation of the ACE2/Ang (1-7)/MasR signaling pathway or inhibition of the ACE/Ang II/AT1R pathway may be alternative treatments for novel coronavirus. Treatment with rhACE2 is the direct approach to regulate the ACE2/Ang (1-7)/MasR axis. A few pilot clinical studies have been conducted using rhACE2 in ARDS, and sepsis. For example, Monteil found that clinical grade recombinant human soluble ACE2 can significantly block early stages of SARS-CoV-2 infections(Monteil *et al.* , 2020). In a pilot clinical trial (NCT01597635)(Khan *et al.* , 2017), patients with ARDS treated with rhACE2 exhibited decreased plasma Ang II and elevated plasma Ang (1-7). An additional pilot study (NCT101884051) investigated the effects of rhACE2 in pulmonary arterial hypertension which is found that the ACE2 activity reduced(Hemnes *et al.* , 2018). The pharmacokinetics and pharmacodynamics of soluble rhACE2 treatment in healthy volunteers showed increased Ang (1-7) with no effect on blood pressure and heart rate (NCT00886353)(Haschke *et al.* , 2013). These clinical trials showed treatment with rhACE2 may improve excessive inflammation via activation of the ACE2/Ang (1-7)/Mas signaling pathway. Interestingly, treatment with rhACE2 may be completed with SARS-Cov-2 for ACE2, thus reduced the susceptibility of ACE2 binding to the virus.

5.3 Rebalancing the RAAS axes by ACEI/ARB

In addition to the treatment with rhACE2, ACEI and ARB can inhibit the ACE/Ang II/AT1R axis, leading to alleviation of inflammation, fibrosis, hypertension and so on. Since ACEI/ARB treatment up-regulates ACE2 expression, there are some controversy about the treatment of ACEI/ARBs in the infected patients coexist with cardiovascular disease. Fang(Fang *et al.* , 2020) and Diaz(Diaz, 2020) both hypothesized patients treated with ACEI/ARBs are at higher risk for SARS-CoV-2 infection due to up-regulation of ACE2 induced by ACEI/ARB, which would enhance viral binding and uptake, while there is no scientific evidence to demonstrate their prediction. On the contrary, someone insisted that ACEI/ARB may be beneficial in COVID-19 therapy, because up-regulation of the ACE2/Ang (1-7)/Mas axis contributes to relieve excessive inflammation(Bai *et al.* , 2016; Igase *et al.* , 2005; Ishiyama *et al.* , 2004). A retrospective study found that continuous administration of ACEI in hospital can reduce the mortality and intubation rate of patients with common viral pneumonia(Henry *et al.* , 2018). However, ACEI was divided into water soluble (captopril and lisinopril) and liposoluble (fosinopril). Some studies found that only lipophilic ACEI can reduce mortality of pneumonia patients. Long term use of lipophilic perindopril can significantly reduce the risk of pneumonia in patients with stroke and hypertension, but also significantly reduce the risk of pneumonia in elderly patients with hypertension(Mortensen *et al.* , 2008). It may be related to the drug characteristics that lipophilic ACEI can inhibit the RAAS activity more effectively, theoretically help to rebuild the balance of RAAS. Meanwhile, use of ARBs can delay the progression of ARDS(Raiden *et al.* , 2002), which may play a role in inhibiting ACE/Ang II/AT1R axis activity and neutrophil activation. Recent findings also demonstrate that patients treated with ARB (Olmesartan) had increased secretion of urinary ACE2, likely from an upregulation mechanism, although unclear(Furuhashi *et al.* , 2015). A new study assessed the impact of ACEI and ARBs treatment on the severity and prognosis in 1128 COVID-19 patients with hypertension. In this multi-center study, they compared the mortality rate between ACEI/ARB group and non-ACEI/ARB group and subsequently found that patients treatment with ACEI/ARBs were associated with lower risk of all-cause mortality compared with ACE/ARB non-users among hospitalized COVID-19 patients(Zhang *et al.* , 2020). Meanwhile, the other smaller retrospective study noted that hypertensive COVID-19 patients receiving ACEI or ARB treatment had a lower rate of severe COVID-19 diseases, a trend toward reduced blood levels of Interleukin-6 plus reduced viral load and increased counts of cytotoxic T cells(Meng *et al.* , 2020). Furthermore, one preliminary study examined 28 patients with severe COVID-19 and 18 patients with mild disease, all of whom also had hypertension, ARB use could reduce the higher risk of severe COVID-19 disease, morbidity and mortality, the result that contradicts the hypothesis that ACEI/ARB use is not benefit(Liu *et al.* , 2020).

Although there is no evidence to support the treatment with ACEI/ARBs could enhance the susceptibility of SARS-CoV-2, withdrawal of ACEI/ARB is not benefit for the patients with cardiovascular diseases. COVID-19 is particularly severe in patients with underlying cardiovascular diseases(Guan *et al.* , 2020), and in many

of these patients, active myocardial injury(Arentz *et al.* , 2020) and cardiomyopathy develop during the course of illness. Among patients with chronic symptomatic heart failure, withdrawal of quinapril resulted in a progressive decline in clinical status(Wang *et al.* , 2020). Meanwhile, among asymptomatic patients with heart failure with recovered left ventricular ejection fraction, withdrawal of RAAS inhibitors resulted in rapid relapse of dilated cardiomyopathy(Halliday *et al.* , 2019). These evidences show among COVID-19 patients with cardiovascular diseases may higher risks after withdrawal of ACEI/ARB. Several cardiology associations released an official statement regarding the treatment of ACEI/ARB for COVID-19 patients. Actually, for those high-risk patients, individualized treatment decisions regarding the maintenance of RAAS inhibitors that should be guided by the hemodynamic status, and clinical stability.

5.4 Rebalancing the RAAS axes by Ang (1-7)

Our previous studies showed that Ang (1-7) exerts inhibitory effects on inflammation and oxidation via activating the ACE2/Ang (1-7)/MasR axis and inhibiting the ACE/Ang II/AT1R axis(Cai *et al.* , 2016; Meng *et al.* , 2015). Ang (1-7)-induced signaling have been clarified in several aspects. The increase in NO release is the most classic aspect related to the protective role of Ang (1-7) and includes calcium-independent pathways and the posttranslational regulation of endothelial nitric oxide synthase (eNOS)(Sampaio *et al.* , 2007). Ang (1-7) additionally decreases TGF- β /NF- κ B signaling, a key pathway in inflammation(Marcus *et al.* , 2013). For example, Ang (1-7) significantly attenuated the peritonitis-induced sepsis via suppressing inflammation and apoptosis(Tsai *et al.* , 2018). Meanwhile, upregulation the ACE2/Ang (1-7)/MasR axis could ameliorate sepsis-induced acute lung injury(Chen *et al.* , 2019). Furthermore, administration of Ang (1-7) significantly reduced the neutrophil recruitment and modulated the inflammation in IBD(Khajjah *et al.* , 2017). Additionally, Ang (1-7) has been proved that pretreatment could attenuate the Ang II-induced renal injury via Mas receptor(Kim *et al.* , 2015). Furthermore, the loose interstitial fibrosis was presented in lung tissue of COVID-19 patient, indicated the clinician should pay more attention to the pulmonary fibrosis(Zhang *et al.* , 2020). In coincidence, Ang (1-7) also exerts protective effects against fibrosis. We found that Ang (1-7) alleviated pulmonary fibrosis by inhibiting NOX4-derived ROS-mediated RhoA/Rock pathway(Meng *et al.* , 2015). The similar effects could be found in liver fibrosis(Cai *et al.* , 2016). The above compelling evidence indicated that the Ang (1-7) may represent novel therapeutic approach in the treatment of COVID-19.

5.5 Rebalancing the RAAS axes by alamandine

Alamandine, a novel member of the RAAS, is similar to Ang (1-7), with one amino acid difference. Alamandine has an Ala which replaces Asp at position 1 of Ang (1-7)(Villela *et al.* , 2014). Alamandine binds to the Mas-related G protein-coupled receptor (MrgD), which exerts anti-inflammation and anti-fibrosis via up-regulating of the ACE2/alamandine/MrgD axis(Huang *et al.* , 2020). Accumulating evidences have demonstrated the alamandine has a profound effect in many diseases, including cardiomyopathy, hypertension and so on(Oliveira *et al.* , 2019; Paquette *et al.* , 2018). For example, administration of alamandine could alleviate the inflammation by decreasing the infiltration of neutrophils and regulating macrophage polarization(de Carvalho *et al.* , 2019). Meanwhile, alamandine protected rat from myocardial injury by inhibiting NF- κ B pathway(Song *et al.* , 2019). Moreover, alamandine produced endothelial-dependent vasorelaxation in aortic rings of mice and rats(Lautner *et al.* , 2013) and attenuated arterial remodeling(de Souza-Neto *et al.* , 2019). Interestingly, alamandine could attenuate hepatic fibrosis by regulating autophagy(Huang *et al.* , 2020). Furthermore, alamandine level is also detectable in human plasma and elevated in patients with chronic renal failure(Hrenak *et al.* , 2016). These experimental researches are not enough to support that alamandine could be a therapeutic approach, while the protective effects of alamandine should not be ignored.

6. Summary

According to above compelling evidences, we speculated that consumption of ACE2 would enhance the ACE/Ang II/AT1R axis but inhibit the ACE2/Ang (1-7)/MasR axis, which resulted in imbalance of RAAS, and thus increased the inflammatory response. Rebalancing the RAAS may be the therapeutic and preventive

strategy for COVID-19 patients. Interventions for rebalancing the RAAS include the treatment of rhACE2, ACEI/ARB, Ang (1-7), alamandine and so on. These interventions may upregulate ACE2 expression, while there is no evidence to support the possibility of increasing the susceptibility of SARS-CoV-2. Nevertheless, the experimental findings have demonstrated that upregulating ACE2 may shift the balance of RAAS axes from ACE/Ang II/AT1R axis to the ACE2/Ang (1-7)/Mas axis. Meanwhile, lots of clinical trials have demonstrated rebalancing the RAAS does play a protective role in improvement of inflammation. Therefore, we discuss our current understanding about the role of ACE2 in organs injury secondary to the COVID-19, explore the benefit effects of the ACE2/Ang (1-7)/Mas axis, and then make our point that rebalances the RAAS can be the therapeutic and preventive strategy. However, further investigation is required to demonstrate whether these experimental effects could be translated into clinical benefit.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

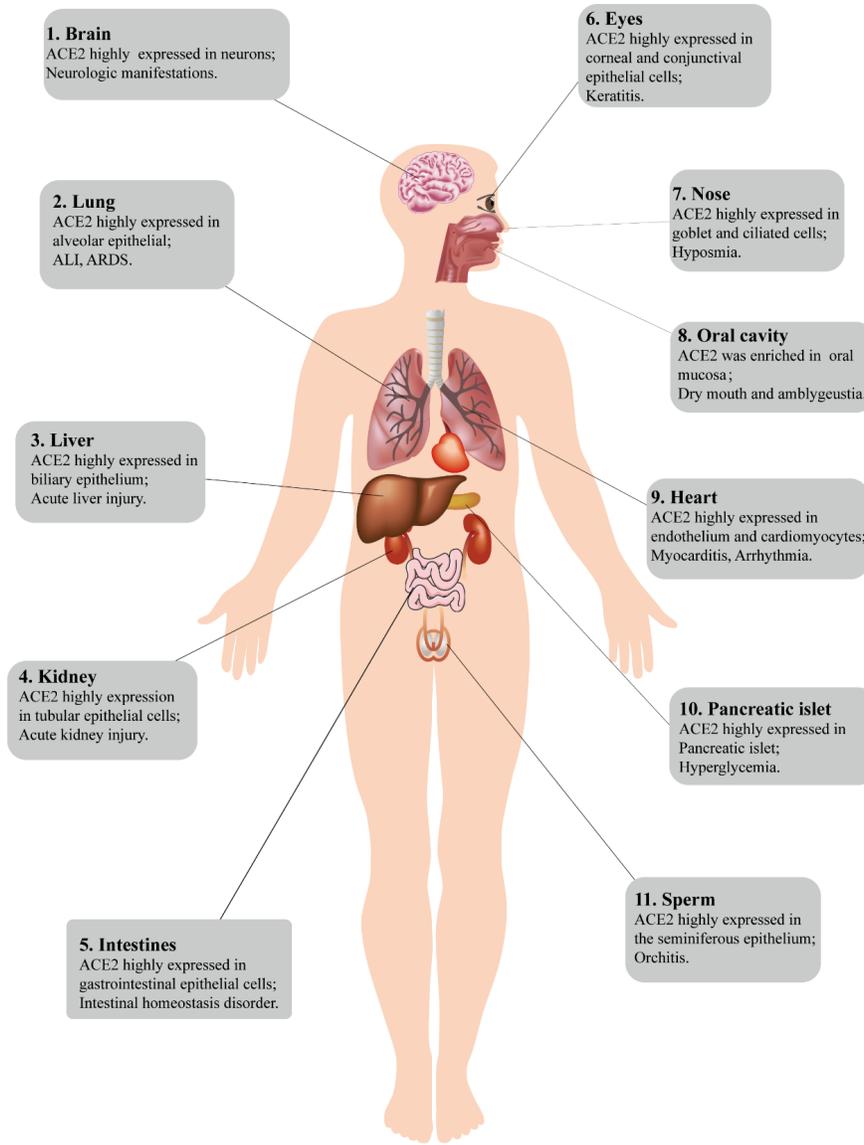


Figure 1 The systemic disorders caused by SARS-CoV-2 infection

Table 1 The infected organs and transmission in COVID-19 patients.

Number	Transmission	Infected organ	Symptoms	Reference
1	Respiratory droplets transmission	Lung	Dry cough, Dyspnea, Pharyngeal pain	Huang <i>et al.</i> , 2020; Wang <i>et al.</i> , 2020
2	Hematogenous transmission	Heart	Arrhythmia, Acute cardiac injury	Huang <i>et al.</i> , 2020; Wang <i>et al.</i> , 2020
3	Hematogenous transmission	Brain	Headache, Dizziness, Anorexia	Chen <i>et al.</i> , 2020; Mao <i>et al.</i> , 2020

Number	Transmission	Infected organ	Symptoms	Reference
4	Hematogenous transmission	Kidney	Proteinuria	Guan <i>et al.</i> , 2020; Huang <i>et al.</i> , 2020; Wang <i>et al.</i> , 2020
5	Hematogenous route	Liver	Abnormal liver enzymes	Liu <i>et al.</i> , 2020; Weber <i>et al.</i> , 2020; Xu <i>et al.</i> , 2020
6	Hematogenous transmission	Pancreatic islet	Glycemic variability	Zhu <i>et al.</i> , 2020
7	Hematogenous transmission	Reproductive system	Serum T to LH ratio decreased	Wang <i>et al.</i> , 2020
8	Contact transmission	Eyes, nose	virus in tears and conjunctival secretions	Xia <i>et al.</i> , 2020; Zhang <i>et al.</i> , 2020
9	Fecal-Oral transmission	Intestine	Diarrhea, Vomiting, Abdominal pain	Lin <i>et al.</i> , 2020; Tang <i>et al.</i> , 2020; Zhang <i>et al.</i> , 2020; Jin <i>et al.</i> , 2020; Young <i>et al.</i> , 2020
10	Fecal-Oral transmission	Oral and saliva	Dry mouth and amblygeusia	Azzi <i>et al.</i> , 2020

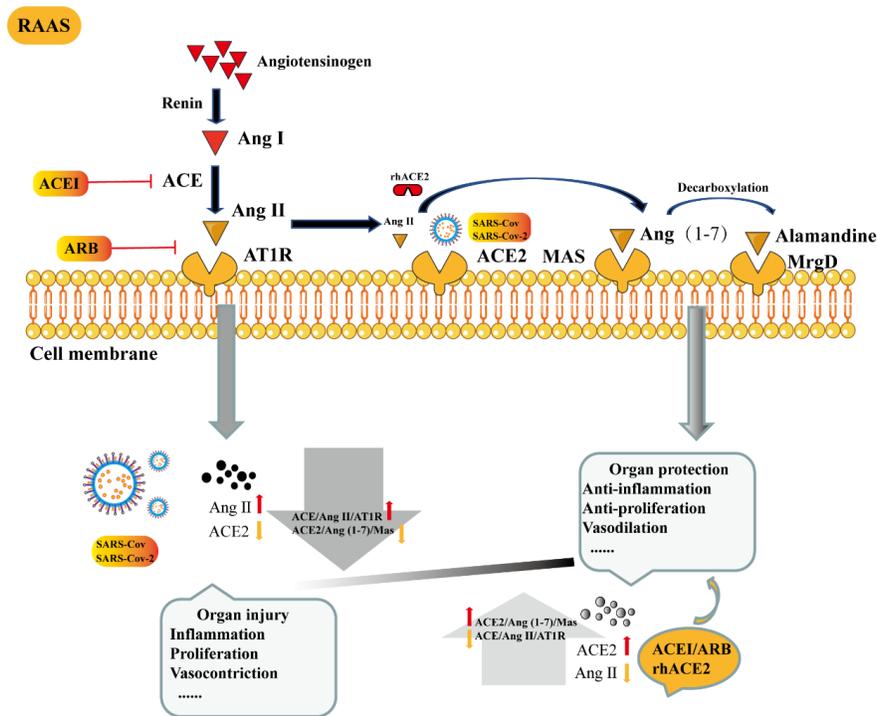


Figure 2 Diagram of renin-angiotensin- aldosterone system (RAAS) and the target of SARS-

Cov-2.

In the diagram, there are two major axes in the RAAS: ACE/Ang II/AT1R axis and ACE2/Ang (1-7)/Mas axis; ACE2 is the key modulator in the RAAS, which plays a key importance in maintaining blood pressure homeostasis, containing fluid and salt balance in mammals. However, ACE2 is the receptor for SARS-Cov-2 infection which may lead to the imbalance of RAAS, and subsequently activate the inflammation in various organ.

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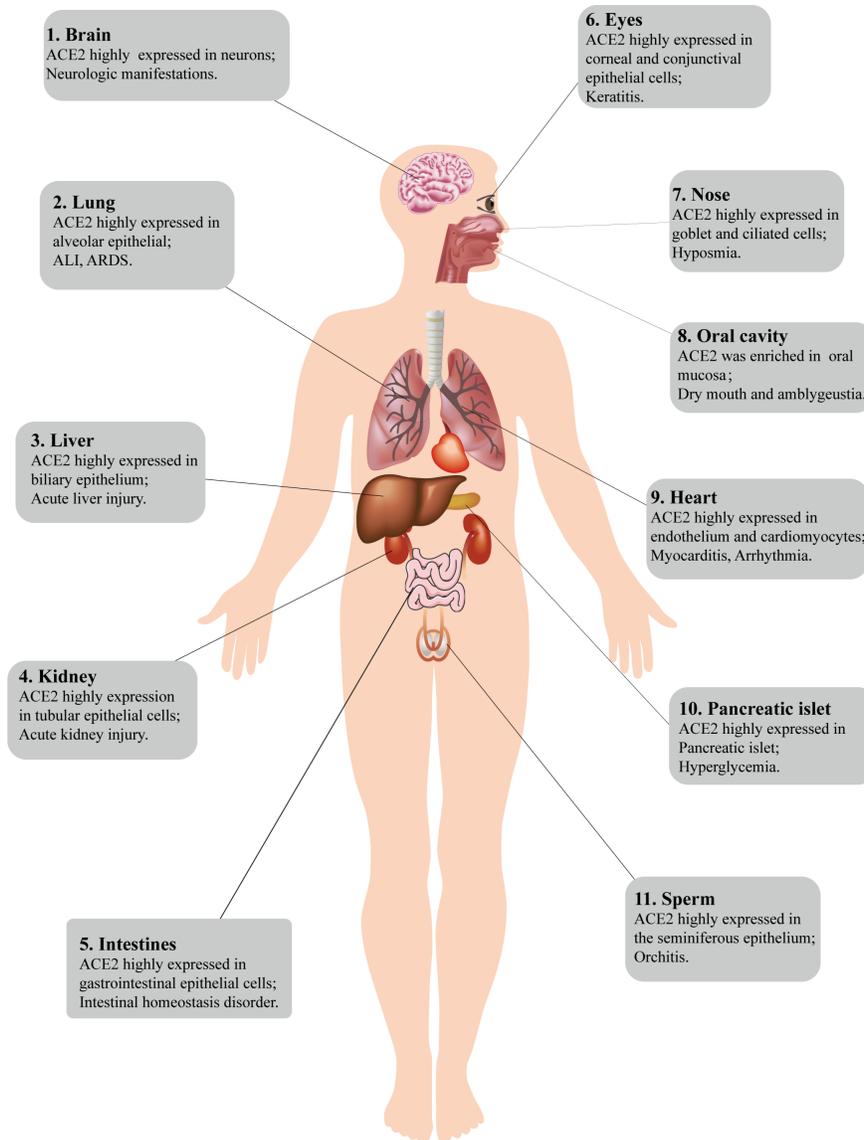
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