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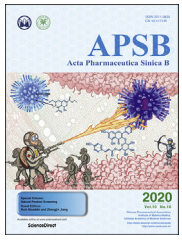

LETTER TO THE EDITOR

Increased plasma ACE2 concentration does not mean increased risk of SARS-CoV-2 infection and increased fatality rate of COVID-19

To the Editor:

More recently, the ongoing dissemination of the novel coronavirus disease 2019 (COVID-19) is posing an unprecedented threat to global healthcare systems[1,2]. Since the outbreak, COVID-19 has escalated to well over 24,775,000 cases and caused over 837,000 deaths worldwide by August 29, 2020. Angiotensin converting enzyme 2 (ACE2) has garnered widespread interest as the receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative virus of COVID-19 pandemic, providing a critical link among COVID-19, inflammatory storm, ACE2 and cardiovascular disease[1,2]. Thus, this brings controversy and challenge to clinical practice: does increased ACE2 level mean increased risk of SARS-CoV-2 infection and increased fatality rate of COVID-19. The latest view from Sama et al.[1] was that higher plasma ACE2 concentrations in patients with heart failure might explain for the higher incidence and fatality rate of COVID-19 in men[1]. They demonstrated that the strongest predictor of elevated concentrations of ACE2 was male sex in both cohorts, and use of ACE inhibitors (ACEI) or angiotensin (Ang) receptor blockers (ARB) was not an independent predictor of plasma ACE2 in the index cohort while ACEI and ARB use were independent predictors of lower plasma ACE2 in the validation cohort[1]. Therefore, they concluded that higher plasma ACE2 concentrations might explain the higher incidence and fatality of COVID-19 in men, and ACEI or ARB could not increase the vulnerability for the COVID-19 through increased plasma ACE2 concentrations.

Here, we put forward a completely different perspective on the causal relationship between ACE2 and SARS-CoV-2 infection and aggravation of COVID-19. Firstly, we think that the conclusions drawn from the results of the two cohorts in this article are one-sided viewpoint in some respects, likely contributing to the unreasonable speculation. Previous research evidence indicates that increased ACE2 levels in lung tissues are associated with the vulnerability of SARS-CoV-2, and ACE2 is proved to be highly expressed in secretory cells in the lung epithelium[3], the wide surface of alveolar epithelial cells might explain for the vulnerability of this organ to SARS-CoV-2 invasion. However, it is inappropriate to speculate a higher tissue expression of ACE2 according to a higher plasma ACE2 concentration since ACE2 is mainly a membrane-anchored enzyme. The view from Sama et al.[1] was that male sex was the strongest predictor of elevated concentrations of circulating ACE2, while Smith et al.[3] demonstrated that ACE2 levels in mammalian lungs did not vary by age or sex. This discrepancy reflected the inconsistency between circulating ACE2 and tissue ACE2 laterally. Furthermore, it is of great significance to distinguish the concepts of different forms of ACE2 and explore the potential relations within them. The membrane-bound ACE2 (mACE2) serves as the major SARS-CoV-2 receptor *via* attaching to the viral Spike protein thus directly mediates the endocytosis of the virus[2]. Soluble ACE2 (sACE2) is produced by the hydrolysis and shedding from mACE2, small amounts of the sACE2 normally exists in circulating blood[4]. Both the mACE2 and sACE2 could catalyze Ang II conversion to protective peptide Ang-(1−7) which, by virtue of its actions on the Mas receptor, opposes the molecular and cellular effects of Ang II[2,5]. Remarkably, recombinant human ACE2 (rhACE2), sACE2, ACE2-Fc, and ACE2-Ig are thought to be promising therapeutic approaches for COVID-19 patients with SARS-CoV-2 infection through competitive inhibiting the binding of viral Spike protein to mACE2[4].

Secondly, there is currently no conclusive evidence that increased ACE2 level will lead to increased susceptibility for SARS-CoV-2 infection and aggravation of COVID-19. It is reported that ACE2 expression is higher in kidney and ileum than that in lung, but both kidney and ileum are not at higher risk of SARS-CoV-2 infection than that of lung, indicating other complicated mechanisms might be involved in the vulnerability of SARS-CoV-2 and COVID-19[2,6,7]. Zou et al.[8] identified the organs with high and low vulnerability according to ACE2 expression and constructed a risk map indicating the vulnerability of different organs to COVID-19 infection inconsistent with the distribution of ACE2. In addition to ACE2 receptor, a disintegrin and metallopeptidase domain 17 (ADAM17), transmembrane protease serine 2 (TMPRSS2), and host molecules such as CD147, GRP78, furin, cathepsin B and cathepsin L may mediate the viral binding and entry processes of SARS-CoV-2 infection (Fig. 1)[2,6]. Notably, a circulating sACE2 ectodomain can be shed following proteolytic cleavage by ADAM17 resulting in loss of ACE2 from the cell

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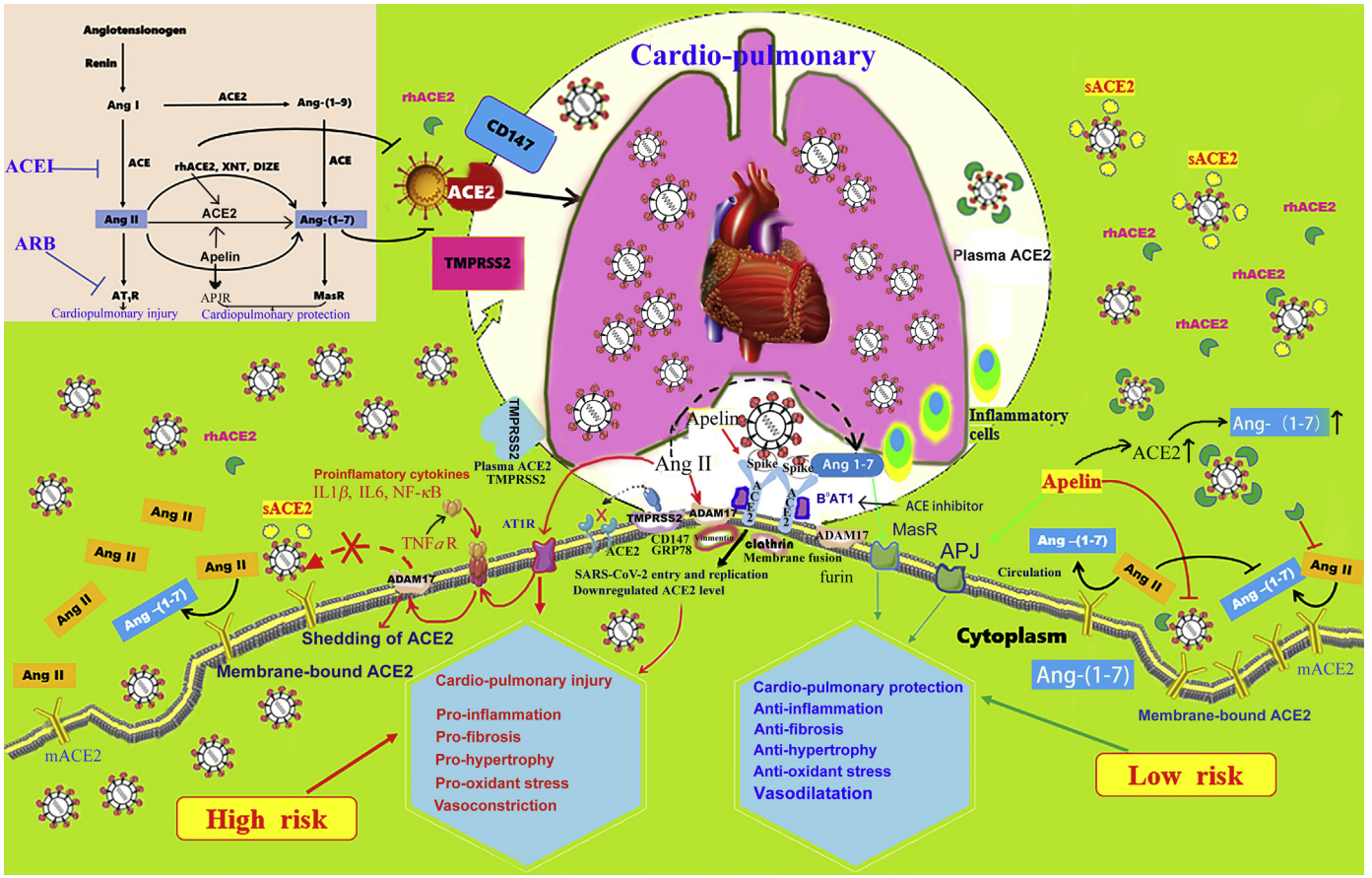

Figure 1 A model for the process of SARS-CoV-2 entering host cells in the lungs and heart and the relationship among ACE2, the RAAS, the apelin-APJ system and SARS-CoV-2 in the pathogenesis of COVID-19. In addition to ACE2 receptor, ADAM17, TMPRSS2, CD147, GRP78, furin, cathepsin B and cathepsin L may mediate the viral binding and entry processes of SARS-CoV-2 infection. Membrane-bound ACE2 (mACE2) catalyzes Ang II conversion to protective peptide Ang-(1−7) and also serves as the major SARS-CoV-2 receptor, directly mediating the endocytosis of SARS-CoV-2 *via* binding to the Spike protein. ACE2-mediated cardiopulmonary protection is lost following endocytosis of the enzyme along with SARS-CoV-2 viral particles. Ang II level elevates with increased activity of AT_1R and further increases ADAM17 activity at the cost of the ACE2/Ang-(1−7)−Mas axis and apelin−APJ axis driven pathways leading to adverse fibrosis, hypertrophy, increased ROS generation, vasoconstriction, and inflammation. The resultant cell-surface downregulation of ACE2 after SARS-CoV-2 infection contributes to the ongoing cardio-pulmonary damage and a cytokine storm due to lower Ang-(1−7)/Ang II ratio (left). Importantly, recombinant human ACE2 (rhACE2) and soluble ACE2 (sACE2) may act as a virus trap and inactivator for SARS-CoV-2 along with higher Ang-(1−7)/Ang II ratio (right). Thus, rhACE2, sACE2, ACE2-Fc, ACE2-Ig, apelin and APJ agonists are currently considered as potentially therapeutic options for COVID-19 patients with SARS-CoV-2.

Table 1 The clinical and preclinical researches of ACE2 and the RAAS in cardio-pulmonary diseases.

Experimental model/population	Clinical/experimental intervention	Effect and observation	Ref.
Clinical researches			
Patients with COVID-19 (n = 12), healthy controls (n = 8)	−	↑Circulating Ang II level in patients with SARS-CoV-2 infection (linearly associated with viral load and lung injury)	9
Postmortem autopsy heart samples of patients with SARS-CoV infection (n = 20)	−	↓Myocardial ACE2 levels in patients with SARS-CoV infection ↑Myocardial inflammation and fibrosis	10
COVID-19 patients with hypertension: ACEI/ARB group (n = 188); non-ACEI/ARB group (n = 940)	ACEI/ARB	↓Risk of all-cause mortality with inpatient use of ACEI/ARB among hospitalized patients with SARS-CoV-2 infection	11
Phase II trial of rhACE2 in patients with chronic HF (n = 59), acute HF (n = 42), healthy controls (n = 36)	rhACE2	↓Plasma Ang II levels; HF symptoms and hospitalization ↑Plasma Ang-(1−7) levels; the Ang-(1−7)/Ang II ratio	12
Phase II trial of rhACE2 in patients with ARDS (n = 60); a randomized, double-blind, placebo-controlled investigation	rhACE2 (GSK2586881)	↑Plasma Ang-(1−7) levels; surfactant protein D; ↓Proinflammatory factor interleukin-6; ↓Plasma Ang II levels	13
PAH patients (n = 11) and healthy controls (n = 8) in observational studies; PAH patients (n = 5) in pilot trail	rhACE2 (GSK2586881)	↓Plasma ACE2 activity; inflammation ↑Cardiac output; plasma SOD2 level	14
Preclinical researches			
Ang II-induced hypertensive mice; TAC-induced HF mice	rhACE2	↓Diastolic dysfunction; myocardial fibrosis; inflammation ↓Plasma and myocardial Ang II levels; myocardial injury; ROS production ↑Plasma Ang (1−7) levels; the Ang-(1-7)/Ang II ratio	15
AngII-infused HF mice	rhACE2-Fc	↓Plasma Ang II levels and blood pressure levels ↓Cardiac hypertrophy; albuminuria; cardiorenal fibrosis	16
Ang II- and TAC-induced HF mice	rhACE2	↓Myocardial hypertrophy; ROS production; ↓Cardiac fibrosis; pro-fibrotic gene expression	17
SHR	rhACE2	↓Myocardial NADPH oxidase activity; oxidative injury ↓Cardiorenal fibrosis; cardiac dysfunction	18
BALB/c mice	ACE2-Ig; mACE2-Ig	Both ACE2-Ig and mACE2-Ig exhibit potent inhibitory activity against SARS-CoV and SARS-CoV-2	19
Vero-E6 cells and human capillary organoids with SARS-CoV-2	hrsACE2	HrsACE2 could inhibit SARS-CoV-2 infection of Vero-E6 cells and human capillary organoids in a dose dependent manner	20

−Not applicable.

ACE2, angiotensin converting enzyme 2; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARDS, acute respiratory distress syndrome; Ang, angiotensin; HF, heart failure; ROS, reactive oxygen species; rhACE2: recombinant human ACE2; RAAS, the renin−angiotensin−aldosterone system; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SHR, spontaneous hypertensive rat; TAC, transverse aortic constriction; hrsACE2, human recombinant soluble ACE2.

surface while TMPRSS2 could activate spike protein of SARS-CoV-2 and cleave the C-terminal segment of ACE2 (Fig. 1), thus enhancing the ability of virus spike protein to enter host cells[2,6]. Furthermore, increased ACE2 could bind to amino acid transporter B°AT1 and overlay the sites where ACE2 is cleaved by TMPRSS2 (Fig. 1)[2]. The expression level of B°AT1 in lung tissue is lower than that in intestine and kidney, which may be one of the reasons why SARS-CoV-2 is prone to infect the lung rather than the intestine and kidney[2]. ACE2 binding virus requires various co-factors, co-receptors and related signal molecules in the complicated infective process of SARS-CoV-2. Thus, ACE2 is not the only factor affecting infection of SARS-CoV-2 during COVID-19 pandemic and increased plasma ACE2 does not completely reflect increased risk of SARS-CoV-2 infection.

Thirdly, the major contributor to progressively worsened systemic manifestations of COVID-19 was due to an imbalance of the Ang-(1−7)/Ang II through a loss of functional tissue ACE2 instead of elevated ACE2 level, thus leading to severe inflammatory storm[2]. Intriguingly, circulating Ang II level was obviously elevated in COVID-19 patients with lung injury (Table 1)[9] which further upregulates ADAM-17 activity by interacting with AT1 receptors, leading to more shedding of ACE2 and thereby

accelerating renin-angiotensin-aldosterone system (RAAS)-mediated injury including severe cardiopulmonary damage (Fig. 1)[2]. In fact, myocardial ACE2 level was downregulated in patients with SARS-CoV infection with enhanced myocardial inflammation and fibrosis (Table 1)[10]. Among hospitalized patients with COVID-19 and coexisting hypertension, inpatient use of ACEI/ARB was associated with lower risk of all-cause mortality compared with ACEI/ARB nonusers (Table 1)[11]. Patients with heart failure have elevated plasma ACE2 level due to increased shedding of tissue ACE2, which may predispose them to the RAAS imbalance mediated by SARS-CoV-2 that further depletes ACE2-mediated protection in heart and lung[2]. Increased sACE2 activity in patients with heart failure indicates an overactive cardioprotective arm of the RAAS to counteract detrimental effects of Ang II[2]. Importantly, in pre-clinical models and clinical populations (Table 1), use of rhACE2 in patients with hypertension, heart failure, acute respiratory distress syndrome and acute lung injury led to a prompt increase in the Ang (1−7)/Ang II ratio and improvement of pathological hypertrophy, cardiopulmonary fibrosis, inflammation, oxidant injury and heart dysfunction, also reflecting ACE2-mediated cardiopulmonary protective impacts[2,12−19]. Furthermore, clinical grade recombinant human sACE2 reduced SARS-CoV-2 recovery from Vero cells, human blood vessel organoids and human kidney organoids, implying that sACE2 can significantly block early stages of SARS-CoV-2 infections[20]. Besides of Ang II, ACE2 also could hydrolyze apelin-13 to apelin-12 which binds to APJ receptor and mediates cardiovascular protective effects such as vasodilation and positive inotropic action (Fig. 1)[21]. Apelin and its receptor APJ agonists are of great potential treatment for COVID-19 through blockade of the RAAS and upregulation of ACE2, which is a key mechanism to inhibit excessive ACE and the Ang II−AT1 signaling activation[22,23]. Exogenous apelin/ACE2 signaling could improve lung injury and alleviate cardiovascular complications through enhancing Ang (1−7)/Ang-II ratio and blocking the Ang II/AT1-mediated prooxidant, pro-inflammatory, pro-hypertrophic, and pro-fibrotic actions (Fig. 1)[21−23].

Collectively, ACE2 has trivalent function: a receptor for SARS-CoV-2, a negative regulator of the RAAS and a partner for amino acid transporters B°AT1. Increased ACE2 concentration does not mean increased risk of SARS-CoV-2 infection and increased cardiovascular comorbidities in patients with greater incidence and fatality rate of COVID-19. Notably, rhACE2, sACE2, ACE2-Fc, ACE2-Ig, Apelin and APJ agonists are currently being considered as potential therapeutic options in treatment of COVID-19.

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Author contributions

All authors collected the data and discussed the article. Xueting Li and Ying Liu wrote the manuscript. Juanjuan Song discussed the content of manuscript and provided some advice. Jiuchang Zhong designed and wrote this manuscript, reviewed and edited it before submission.

Conflicts of interest

The authors declare no conflicts of interest.

References

1 Sama IE, Ravera A, Santema BT, van Goor H, Ter Maaten JM, Cleland JG, et al. Circulating plasma concentrations of angiotensin-converting enzyme 2 in men and women with heart failure and effects of renin-angiotensin-aldosterone inhibitors. *Eur Heart J* 2020;**41**:1810−7.
2 Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. *Circ Res* 2020;**126**:1456−74.
3 Smith JC, Sausville EL, Girish V, Yuan ML, Vasudevan A, John KM, et al. Cigarette smoke exposure and inflammatory signaling increase the expression of the SARS-CoV-2 receptor ACE2 in the respiratory tract. *Dev Cell* 2020;**53**:514−29. e3.
4 Batlle D, Wysocki J, Satchell K. Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy?. *Clin Sci (Lond)* 2020;**134**:543−5.
5 Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur J Intern Med* 2020;**76**:14−20.
6 Aguiar JA, Tremblay BJ, Mansfield MJ, Woody O, Lobb B, Banerjee A, et al. Gene expression and in situ protein profiling of candidate SARS-CoV-2 receptors in human airway epithelial cells and lung tissue. *Eur Respir J* 2020;**56**:2001123.
7 Bian J, Zhao R, Zhai S, Li Z. Letter to the editor: anti-RAS drugs and SARS-CoV-2 infection. *Acta Pharm Sin B* 2020;**10**:1251−2.
8 Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front Med* 2020;**14**:185−92.
9 Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci* 2020;**63**:364−74.
10 Oudit GY, Kassiri Z, Jiang C, Liu PP, Poutanen SM, Penninger JM, Butany J. SARS-coronavirus modulation of myocardial ACE2 expression and inflammation in patients with SARS. *Eur J Clin Invest* 2009;**39**:618−25.
11 Zhang P, Zhu L, Cai J, Lei F, Qin JJ, Xie J, et al. Association of inpatient use of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers with mortality among patients with hypertension hospitalized with COVID-19. *Circ Res* 2020;**126**:1671−81.
12 Basu R, Poglitsch M, Yogasundaram H, Thomas J, Rowe BH, Oudit GY. Roles of angiotensin peptides and recombinant human ACE2 in heart failure. *J Am Coll Cardiol* 2017;**69**:805−19.
13 Khan A, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit Care* 2017;**21**:234.
14 Hemnes AR, Rathinasabapathy A, Austin EA, Brittain EL, Carrier EJ, Chen X, et al. A potential therapeutic role for angiotensin-converting enzyme 2 in human pulmonary arterial hypertension. *Eur Respir J* 2018;**51**:1702638.
15 Zhong J, Basu R, Guo D, Chow FL, Byrns S, Schuster M, et al. Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. *Circulation* 2010;**122**:717−28.
16 Liu P, Wysocki J, Souma T, Ye M, Ramirez V, Zhou B, et al. Novel ACE2-Fc chimeric fusion provides long-lasting hypertension control and organ protection in mouse models of systemic renin angiotensin system activation. *Kidney Int* 2018;**94**:114−25.
17 Epelman S, Tang WH, Chen SY, Van Lente F, Francis GS, Sen S. Detection of soluble angiotensin-converting enzyme 2 in heart failure: insights into the endogenous counter-regulatory pathway of the renin-angiotensin-aldosterone system. *J Am Coll Cardiol* 2008;**52**:750−4.

18 Lo J, Patel VB, Wang Z, Levasseur J, Kaufman S, Penninger JM, et al. Angiotensin-converting enzyme 2 antagonizes angiotensin II-induced pressor response and NADPH oxidase activation in Wistar-Kyoto rats and spontaneously hypertensive rats. *Exp Physiol* 2013;**98**:109−22.
19 Lei C, Qian K, Li T, Zhang S, Fu W, Ding M, et al. Neutralization of SARS-CoV-2 spike pseudotyped virus by recombinant ACE2-Ig. *Nat Commun* 2020;**11**:2070.
20 Monteil V, Kwon H, Prado P, Hagelkrüys A, Wimmer RA, Stahl M, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell* 2020;**181**:905−13. e7.
21 Robinson EL, Alkass K, Bergmann O, Maguire JJ, Roderick HL, Davenport AP. Genes encoding ACE2, TMPRSS2 and related proteins mediating SARS-CoV-2 viral entry are upregulated with age in human cardiomyocytes. *J Mol Cell Cardiol* 2020;**147**:88−91.
22 Zhang ZZ, Wang W, Jin HY, Chen X, Cheng YW, Xu YL, et al. Apelin is a negative regulator of angiotensin II-mediated adverse myocardial remodeling and dysfunction. *Hypertension* 2017;**70**:1165−75.
23 Saeedi Saravi SS, Beer JH. Apelin-potential therapy for COVID-19?. *J Mol Cell Cardiol* 2020;**145**:84−7.

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