Hypothesis

Possible Involvement of Autophagy or Apoptosis Dysregulation in Infection with COVID-19 Virus as the Main Cause of Mortality: Hypothetical Function of ACE-2/Mas / Ang (1-7) Signaling Pathway and Proposal of a Post-infection Treatment Strategy

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Virus infection pandemic COVID-19, which is from the coronavirus family, is becoming a global concern and an emergency for public health in 2019 and 2020 *[1, 2]*. According to current evidence, coronavirus infection causes moderate respiratory tract disorder and also leads to cardiovascular dysfunction when in extreme cases of coronavirus family infections such as SARS (Severe Acute Respiratory Syndrome), Middle East respiratory syndrome coronavirus (MERS) and COVID-19 (coronavirus disease-19) death may occur because all these forms of coronavirus have long-term cardiovascular and respiratory sequelae. However, the exact mechanism of these lethal processes and the signaling pathway involved in the cardiovascular system remains uncertain, and unfortunately, not enough information is available in this regard *[2-5]*. According to some recent study, angiotensin-converting enzyme 2 (ACE2) is one of the key targets of infection with COVID-19 and can also be used as a host receptor for SARS and MERS *[3, 6]*. Considering the basic principle of the main mechanism of coronavirus family pathogenesis, all these types of viruses namely SARS, MERS and COVID-19 have used ACE2 (as their host receptor) and can enter their target cells, including pulmonary and cardiovascular cells, through this enzyme *[7, 8]*. It has been shown that the ACE 2 over-expression has increased the risk of coronavirus infection in certain populations and ethnicities *[9, 10]*. Although the data on the COVIID-19 infection process and the role of ACE2 in this process are less than the data on SARS and the MERS pathogenesis process, in a recent study, it has been indicated that human recombinant soluble ACE2 (hrsACE2) blocks COVID-19 growth and can significantly block early stages of COVID-19 infections *[11]*. The results of this study showed that ACE2 inhibition can block infection but would not discuss the role of ACE2 after infection and in reduction of patients' mortality *[11]*. On the other hand, concerning the role of ACE2 in the mortality rate of animal models with SARS infection, it was found that in mice with ACE2-/-, which were knocked down by their ACE2 genes; being

infected with SARS virus is less likely compared to the normal mice (wild type) while mortality rates have been increased and are more than the average *[8, 12]*. The aforementioned data suggest that these ACE2-/- animals are less likely to be infected with SARS and other forms of coronaviruses, but if they were infected, their mortality rate will be increased *[8, 12]*. Therefore, it can be inferred that ACE2 has a dual complicated role in reducing the risk and the probability of coronavirus infection while its absence increases mortality risk *[8-10]*. On the other hand, based on clinical evidence regarding COVID-19 infection in particular, it was shown that the mortality rate from COVID-19 infection was extremely high in the elderly and patients with underlying heart and respiratory disease *[13, 14]*. Seemingly, one of the main reasons for this increase in the mortality rate of such patients is the reduction of expression of ACE2 *[15, 16]*. ACE2, as a potent cardio-pulmonary protective protein, plays an important role in reducing the cardiovascular incident and can also cause modulation of respiratory system function and performance *[17, 18]*. Angiotensin-converting enzyme 2 (ACE2) induces angiotensin (1-7) [Ang-(1-7)] to be produced from angiotensin-2 (AngII). Several previous studies have shown that the ACE2-Ang-(1-7) axis has a significant function in regulating normal cardiovascular and respiratory physiology and can be targeted as novel therapeutic agents in cardiopulmonary diseases *[17-19]*. They have also shown that ACE2 can inhibit cell death in both cardiac and pulmonary systems which indicate that this protein can constrain apoptosis and autophagy-related signaling pathways and that this mechanism can prevent cell death in various organs *[19-21]*. **Figure 1**. shows an illustration of the role of ACE2 on the signaling pathway for apoptosis and autophagy, and its mechanism for cell death prevention in both cardiac and pulmonary systems. Based on the aforementioned studies on the essential and protective function of ACE2 in the inhibition of cell death, apoptosis, and autophagy in both cardiac and pulmonary

systems, high-risk patients, such as elderly people and patients with respiratory and cardiovascular disease, were hypothesized.

The angiotensin-converting enzyme 2 (ACE2) induces angiotensin (1-7) [Ang-(1-7)] development of angiotensin-2 (AngII). Ang (1-7) causes AT2R, which is the angiotensin receptor type-2. On the other hand, it has been demonstrated that ACE-2/Ang (1-7)/Mas causes B-cell lymphoma 2 (Bcl-2)/Beclin1 or Bcl-2/Bax complex stability and inhibits autophagy and apoptosis in the cardio-respiratory system, respectively. Ang (1-7) can inhibit phosphorylation by the c-Jun N-terminal kinase (JNK). Apoptosis and autophagy are regulated by the complexes of Bcl-2/Beclin1 and Bcl-2/Bax. Bcl-2 is an anti-apoptotic protein that interacts with Beclin1 (the key protein involved in autophagy) and Bax (the main protein involved in autophagy) in an unphosphorylated form. The induction of Bcl-2 phosphorylation after indications of autophagy or apoptosis including dysfunction in the signaling pathway of ACE-2/Mas / Ang (1-7) occurs in patients with the underlying cardio-respiratory disease and activates JNK. It leads to the phosphorylation (inactivation) of Bcl-2 and dissociation of this protein from Beclin1 or **Bcl-2-associated X protein**(Bax) and thus, it causes autophagy or apoptosis in the respiratory and cardiac tissue of the patients and increases their mortality rate. Some indirect evidence indicates that one of the key causes of increased mortality rates in high-risk patients with COVID-19 virus infection is the lower expression and inactivation of ACE-2/Mas / Ang (1-7) in the cardiovascular system, resulting in the activation of JNC / Bcl-2-Beclin1 or JNC / Bcl-2-Bax signaling pathway and the initiation of autophagy or apoptosis and also the death signal (**Figure 1**).

The results show that infection with COVID-19 and other forms of coronaviruses has increased mortality rates and one of the key reasons of the increased mortality rates is a decrease in the expression of ACE2 and an increase in the apoptosis and autophagy cycle that occurred after this decrease in expression. It is also suggested that targeting and/or expression of ACE2 activity, and probably its downstream Ang (1-7)/ Mas axis in the respiratory and cardiovascular system can

protect these systems and inhibit their denial and dysfunction, leading to the decrease in the mortality rates following COVID-19 infection and other types of coronaviruses. However, this therapeutic recommendation (activation of ACE2 or increasing its expression) is useful only after the incident of coronavirus infection and can be useful in protecting the cardiopulmonary system. Whereas, it cannot play a preventive role because increasing the expression of this enzyme will increase the prevalence of the disease. In fact, our therapeutic advice to increase the expression of ACE2 is a post-infection therapy approach and is not a preventive one.

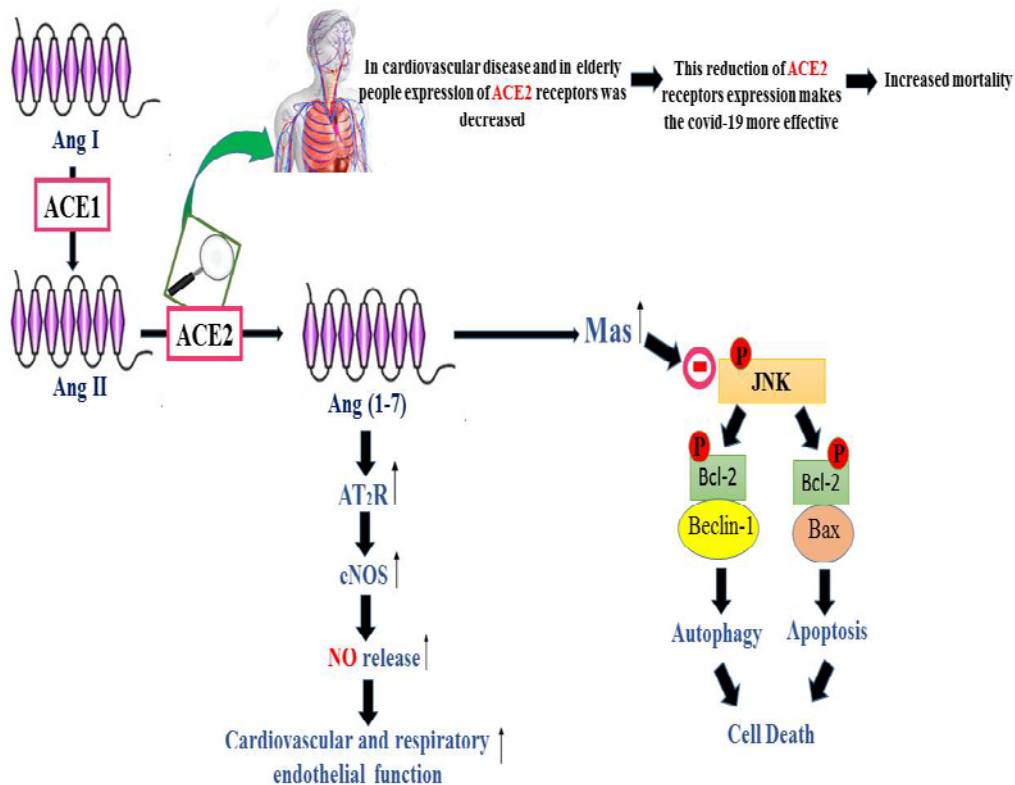**Key words:** COVID-19, ACE-2 / Ang (1-7) / Mas, Apoptosis, Autophagy

Figure 1: The role of ACE2 on the signaling pathway for apoptosis and autophagy, and its mechanism for cell death prevention in both cardiac and pulmonary systems

Conflict of Interest

None declared.

References:

[1] Organization WH. **Infection prevention and control guidance for long-term care facilities in the context of COVID-19: interim guidance, 21 March 2020**. World Health Organization; 2020

[2] Rothan HA, Byrareddy SN. **The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak**. *Journal of Autoimmunity*. 2020:102433

[3] Zheng Y-Y, Ma Y-T, Zhang J-Y, Xie X. **COVID-19 and the cardiovascular system**. *Nature Reviews Cardiology*. 2020:1-2

[4] Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. **Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis**. *International Journal of Infectious Diseases*. 2020

[5] Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. **Pathological findings of COVID-19 associated with acute respiratory distress syndrome**. *The Lancet respiratory medicine*. 2020

[6] Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. **Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target**. *Intensive Care Medicine*. 2020:1-5

[7] Cao Y, Li L, Feng Z, Wan S, Huang P, Sun X, et al. **Comparative genetic analysis of the novel coronavirus (2019-nCoV/SARS-CoV-2) receptor ACE2 in different populations**. *Cell Discovery*. 2020;6:1-4

[8] Yang X-h, Deng W, Tong Z, Liu Y-x, Zhang L-f, Zhu H, et al. **Mice transgenic for human angiotensin-converting enzyme 2 provide a model for SARS coronavirus infection**. *Comparative medicine*. 2007;57:450-9

[9] Hofmann H, Geier M, Marzi A, Krumbiegel M, Peipp M, Fey GH, et al. **Susceptibility to SARS coronavirus S protein-driven infection correlates with expression of angiotensin converting enzyme 2 and infection can be blocked by soluble receptor**. *Biochemical and biophysical research communications*. 2004;319:1216-21

[10] Jia HP, Look DC, Shi L, Hickey M, Pewe L, Netland J, et al. **ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia**. *Journal of virology*. 2005;79:14614-21

[11] 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

[12] Tseng C-TK, Huang C, Newman P, Wang N, Narayanan K, Watts DM, et al. **Severe acute respiratory syndrome coronavirus infection of mice transgenic for the human Angiotensin-converting enzyme 2 virus receptor**. *Journal of virology*. 2007;81:1162-73

[13] Matsushita K, Ding N, Kou M, Hu X, Chen M, Gao Y, et al. **The relationship of COVID-19 severity with cardiovascular disease and its traditional risk factors: A systematic review and meta-analysis**. *medRxiv*. 2020

[14] COVID C, COVID C, COVID C, Chow N, Fleming-Dutra K, Gierke R, et al. **Preliminary Estimates of the Prevalence of Selected Underlying Health Conditions Among Patients with Coronavirus Disease 2019—United States, February 12–March 28, 2020**. *Morbidity and Mortality Weekly Report*. 2020;69:382

[15] Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, et al. **Angiotensin-converting enzyme 2 is an essential regulator of heart function**. *Nature*. 2002;417:822-8

[16] Thomson G. **COVID-19: social distancing, ACE 2 receptors, protease inhibitors and beyond?** *International journal of clinical practice*. 2020:e13503

[17] Oudit GY, Crackower MA, Backx PH, Penninger JM. **The role of ACE2 in cardiovascular physiology**. *Trends in cardiovascular medicine*. 2003;13:93-101

[18] Jia H. **Pulmonary angiotensin-converting enzyme 2 (ACE2) and inflammatory lung disease**. *Shock*. 2016;46:239-48

[19] Yu J, Wu Y, Zhang Y, Zhang L, Ma Q, Luo X. **Role of ACE2-Ang (1-7)-Mas receptor axis in heart failure with preserved ejection fraction with hypertension**. *Zhong nan da xue xue bao Yi xue ban= Journal of Central South University Medical sciences*. 2018;43:738-46

[20] Lin L, Liu X, Xu J, Weng L, Ren J, Ge J, et al. **Mas receptor mediates cardioprotection of angiotensin-(1-7) against Angiotensin II-induced cardiomyocyte autophagy and cardiac remodelling through inhibition of oxidative stress**. *Journal of cellular and molecular medicine*. 2016;20:48-57

[21] Li Y, Cao Y, Zeng Z, Liang M, Xue Y, Xi C, et al. **Angiotensin-converting enzyme 2/angiotensin-(1–7)/Mas axis prevents lipopolysaccharide–induced apoptosis of pulmonary microvascular endothelial cells by inhibiting JNK/NF–κB pathways**. *Scientific reports*. 2015;5:8209