Yin-Yang balance of ACE/ACE2 pathways: the rational for ACE2 pathway inhibitor administration in SARS-CoV-2 patients.

Corresponding author: Loris Zamai, Department of Biomolecular Sciences, via Ca’ le Suore 2, University of Urbino Carlo Bo, 61029 Urbino, Italy.
Tel. (+39) 0722 304319; e-mail: loris.zamai@uniurb.it

Standfirst

The article describes the rational for inhibition of the angiotensin-converting enzyme 2 (ACE2) pathways as specific targets in patients infected by SARS-CoV-2.

Key words: Severe Acute Respiratory Syndrome Coronavirus-2; (soluble) ACE2, eosinophil, asthma, IL-10, IL-6, Lung fibrosis, Ang (1-7), hypoxia, infarction, hypertension.

Introduction

The clinical characteristic and allergy status of 140 patients infected by SARS-CoV-2 has been recently described. Infection is known to produce non-severe and severe symptoms, and in more severe cases, may lead to severe acute respiratory syndrome (SARS) and even death. Elder age and high number of comorbidities were associated with critical patients. Lymphopenia and eosinopenia were observed in most patients, and critical patients have extremely low values of eosinophils, suggesting that eosinopenia may be a potential marker for diagnosis. Moreover, a cytokine storm mainly involving IL-6, IL-8 and IL-10 upregulation is associated with most severe (SARS) cases. Of note, hypertension, an age related disease, was the most common comorbidity, while asthma and other allergic diseases were not reported by any of the 140 infected patients, nor by any patients in other reports. It is generally known that virus infections may increase the risk of allergic disease exacerbation; however, the reported data seem to suggest that the opposite is true. Indeed, the absence, in the examined cohorts, of asthmatic patients suggests that they might be protected from virus induced SARS, whereas pre-existent hypertensive disease and/or pre-existent antihypertensive treatments represent a risk factor for virus infection. Therefore, according to the clinical picture of infected patients, Th2-mediated allergic diseases (usually with high eosinophil counts) may play a protective role against this severe acute respiratory syndrome (usually with low eosinophil counts), while still obscure mechanisms related to hypertensive conditions may exacerbate symptoms.

ACE2 mediated SARS-CoV-2 infection

It is known that SARS-CoV-2 virus shares about 80% sequencing identity with the original SARS-CoV virus. Of note, angiotensin-converting enzyme 2 (ACE2) was identified as a receptor for the spike (S) protein of SARS-CoV after its priming by cellular serine protease TMPRSS2, finally facilitating viral entry into target cells. ACE2 is abundantly expressed in airway epithelial cells and it is believed to play a crucial role in the control of acute lung injury induced by SARS-CoV. Spike-Fc protein treatment (3h) resulted in a downregulation of ACE2 protein expression both in cell lines and in lung cells of mice in vivo, suggesting that ACE2 pathway may be down-regulated during infection. However, the engagement of ACE by spike S protein of SARS-CoV may induce a cellular “protective” ACE2 shedding feedback that does not necessarily mean that the soluble ACE2 is functionally inactive. Indeed, there is evidence that soluble forms of ACE2 are active and even associated to pathological conditions. Moreover, a recent integrative bioinformatics analysis shows that the expression of ACE2 in human bronchial cells infected with SARS-CoV is dramatically increased 24h after infection and

© 2020 by the author(s). Distributed under a Creative Commons CC BY license.
remained at a high level for at least 2 days, suggesting that ACE2 may be involved in a positive feed-back loop post-infection\(^6\). In the same report, it has been shown that expression level of ACE2 in bronchial epithelium obtained by brushing from asthmatic and normal subjects was similar, suggesting that respiratory epithelial cells of healthy subjects and asthmatic patients have similar ability to bind to SARS-CoV-2 through ACE2. Of note, ACE2 was also identified as the receptor for the novel (TMPRSS2 primed) spike protein of SARS-CoV-2\(^3\). Although the role of ACE2 in the pathogenesis of SARS-CoV-2 and in inducing lung injury is still unknown, ACE2 acts similarly to original SARS-CoV through spike (S) protein\(^3\).

Pathological effects of (s)ACE/Ang (1-7)/Mas receptor pathway upregulation and feedback mechanisms of regulation

ACE2 and ACE are key enzymes of the renin-angiotensin system. ACE2 processes angiotensin (Ang) I and II into Ang (1-9) and (1-7), respectively, and it has also other known peptide targets, such as Bradykinin metabolites. Ang (1-7) peptide, opposing the effects of ACE-generated Ang II, has been shown to mediate vasodilatative (hypotension), antiproliferative and apoptotic effects through Mas receptor\(^7,8\).

(s)ACE2 or Ang (1-7) upregulation have been associated to some pathological conditions such as inflammation of the gastrointestinal tract, human cirrhosis, infarction and lung injury/fibrosis\(^5,9-13\). For example, elevated plasma sACE2 activity was associated both with greater severity of myocardial dysfunction and with an independent prediction of adverse clinical events\(^5,9\). Moreover, (s)ACE2 activity and Ang (1-7) concentrations in plasma of patients with inflammatory bowel disease were higher compared to controls\(^10\). In healthy livers, ACE2 is limited to perivenular hepatocytes and endothelial cells, instead in human cirrhosis, ACE2 protein expression is widespread in the hepatic parenchyma. Of interest, human hepatocytes cultured in hypoxic conditions upregulated ACE2 protein expression\(^11\). To this regard, intrapulmonary activation of the ACE2/Ang (1-7)/Mas receptor axis has been described in Ren-2 transgenic rats exposed to chronic hypoxia\(^12\), raising the possibility of a ACE2 upregulation through a positive feedback loop in hypoxia, a condition that finally occurs in SARS patient and that might later sustain SARS independently of virus infection. Interestingly, in vivo administration of Ang-(1-7) alone (in Wistar rats) has been shown to promote morphological lung alterations, extracellular matrix accumulation and inflammatory cytokines (including TNF-alfa, and IL-6), characteristics of lung inflammation in pulmonary fibrosis\(^13\). Moreover, Ang-(1-7) alone either activated the bax/caspase-dependent apoptotic pathway or upregulated NF-kB signaling in lung fibroblasts\(^14\). To this regard, Ang-(1-7) has been also reported to promote eosinophil apoptosis in the lung and in the bronchoalveolar lavage\(^8\). Interestingly, Ang-(1-7)/Mas receptor axis inhibits allergic airway inflammation and eosinophil cell counts in a murine model of asthma, indicating that both an impairment of ACE2 pathway may favor asthma and ACE2 pathway activation can reduces asthma symptoms\(^14\). Moreover, a compound that mimics the angiotensin (Ang)-(1–7) actions has been shown to induce IL-10 upregulation via a Mas receptor-dependent pathway in bronchoalveolar lavage\(^15\). Of note, IL-10 is one of the cytokines downstream ACE2 pathway\(^6\) and together with IL-6 is significantly upregulated in the most severe (SARS) cases\(^2\), suggesting an important correlation between ACE2/Ang (1-7) axis activation and SARS.

Severe acute respiratory syndrome and anti-hypertensive treatments

Correlation of severe (SARS) symptoms with pre-existent hypertension and with age was also described. It is not still clear whether it depends on constitutive hypertensive conditions or/and on anti-hypertensive treatments or on other age-related conditions. To this regard, reduction in ACE2 expression was associated with arterial aging in mice\(^16\) and ACE2 expression is lowered in human renal tissue of hypertensive patients\(^17\). On the other hand, up-regulation of ACE2 and Mas expression was reported in spontaneously hypertensive rats subjected to chronic exercise of moderate intensity\(^18\) and anti-hypertensive ACE inhibitors have been shown to
increase cardiac ACE2 gene expression and cardiac ACE2 activity\textsuperscript{19}. Notably, hypertension, the most frequent comorbidity with SARS-CoV-2, is often treated with ACE inhibitors, suggesting a possible positive correlation that should be avoided to reduce ACE2-mediated viral entry.

**Discussion**

No specific therapeutics are available. Animals immunized with inactivated SARS-CoV vaccines developed a severe (asthma-like) lung eosinophilic immunopathology when challenged with SARS virus, indicating a central role of eosinophil “balanced numbers” in this pathology\textsuperscript{15}. Vaccines might generate antibodies against viral ligand/ACE2 complex that finally blocks ACE2 activity during SARS-Cov virus infection and consequent downstream asthma-like events/symptoms. On the other hand, eosinopenia and cytokine profile (e.g. IL-10) are compatible with downstream events stemming from an excessive ACE2 pathway activation. Altogether these data imply not only that the ACE2 is the “vehicle” of viral entry into the host cells but also that the virus sustains ACE2 pathway activation, finally promoting SARS-induced lung injury.

Since the clinical picture as a whole is consistent with a ACE2 gain of function (likely due to increase of local active forms of soluble ACE2) rather than a ACE2 loss of function, as initially supposed, inhibition of ACE2/ANG (1–7)/Mas receptor axis or other ACE2 pathways to restore ACE/ACE2 balance is desirable. Different strategies can be pursued through ACE2 pathway inhibitors (Dx600, MLN-4760, GL1001, NAAE\textsuperscript{7} and/or Mas receptor antagonist (A-779)\textsuperscript{15} and/or other ACE2 pathways (e.g. involving Bradykinin metabolites or other ACE2 substrates). For example, NAAE demonstrated an antiviral activity\textsuperscript{21} and GL1001 showed to produce an anti-inflammatory activity in a mouse model of colitis\textsuperscript{22}, highlighting the importance of the yin-yang balance of ACE/ACE2 pathways: “in medio stat virtus”.

Unfortunately, to my knowledge, it seems that these inhibitors have not been tested yet in vivo in humans (perhaps because of their potential risk of hypertensive effects); nevertheless, in this exceptionally critical situation, the administration of some of them by aerosol/inhalation might be hopefully considered.

**References**

1. Zhang JJ, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, Akdis CA, Gao YD. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. Allergy. 2020 Feb 19. doi: 10.1111/all.14238.

2. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, Xie C, Ma K, Shang K, Wang W, Tian D-S. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin Infect Dis. Preprint https://doi.org/10.1093/cid/ciaa248

3. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020 Mar 4. pii: 50092-8674(20)30229-4. doi: 10.1016/j.cell.2020.02.052.

4. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat.Med. 2005; 11:875–879.
5. Epelman S, Shrestha K, Troughton RW, Francis GS, Sen S, Klein AL, Tang WH. Soluble angiotensin-converting enzyme 2 in human heart failure: relation with myocardial function and clinical outcomes. J Card Fail. 2009 Sep;15(7):565-71. doi: 10.1016/j.cardfail.2009.01.014. Epub 2009 Mar 17.

6. He X, Zhang L, Ran Q, Xiong A, Wang J, Wu D, Chen F, Li G. Integrative Bioinformatics Analysis Provides Insight into the Molecular Mechanisms of 2019-nCoV. Posted February 05, 2020. medRxiv https://doi.org/10.1101/2020.02.03.2002026

7. Xu P, Sriramula S, Lazartigues E. ACE2/ANG-(1-7)/Mas pathway in the brain: the axis of good. Am J Physiol Regul Integr Comp Physiol. 2011 Apr;300(4):R804-17. doi: 10.1152/ajpregu.00222.2010.

8. Magalhães GS, Barroso LC, Reis AC, Rodrigues-Machado MG, Gregorio JF, Motta-Santos D, et al. Angiotensin-(1–7) Promotes Resolution of Eosinophilic Inflammation in an Experimental Model of Asthma. Front Immunol. 2018; 9:58. Epub 2018/02/13. https://doi.org/10.3389/fimmu.2018.00058

9. Ortiz-Pérez JT, Riera M, Bosch X, De Caralt TM, Perea RJ, Pascual J, Soler MJ. Role of circulating angiotensin converting enzyme 2 in left ventricular remodeling following myocardial infarction: a prospective controlled study. PLoS One. 2013 Apr 22;8(4):e61695. doi: 10.1371/journal.pone.0061695.

10. Garg M, Burrell LM, Velkoska E, Griggs K, Angus PW, Gibson PR, Lubel JS. Upregulation of circulating components of the alternative renin-angiotensin system in inflammatory bowel disease: A pilot study. J Renin Angiotensin Aldosterone Syst. 2015 Sep;16(3):559-69. doi: 10.1177/1470320314521086.

11. Paizis G, Tikellis C, Cooper ME, Schembri JM, Lew RA, Smith Al, Shaw T, Warner FJ, Zuilli A, Burrell LM, Angus PW. Chronic liver injury in rats and humans upregulates the novel enzyme angiotensin converting enzyme 2. Gut. 2005 Dec;54(12):1790-6.

12. Hampl V, Herget J, Bíbová J, Baňasová A, Husková Z, Vaňourková Z, Jíchová Š, Kujal P, Vernerová Z, Sadowski J, Červenka L. Intrapulmonary activation of the angiotensin-converting enzyme type 2/angiotensin 1-7/G-protein-coupled Mas receptor axis attenuates pulmonary hypertension in Ren-2 transgenic rats exposed to chronic hypoxia. Physiol Res. 2015;64(1):25-38.

13. Meng Y, Yu CH, Li W, Li T, Luo W, Huang S, Wu PS, Cai SX, Li X. Angiotensin-converting enzyme 2/angiotensin-(1-7)/Mas axis protects against lung fibrosis by inhibiting the MAPK/NF-κB pathway. Am J Respir Cell Mol Biol. 2014 Apr;50(4):723-36. doi: 10.1165/rcmb.2012-0451OC.

14. El-Hashim AZ, Renno WM, Raghupathy R, Abduo HT, Akhtar S, Benter IF. Angiotensin-(1-7) inhibits allergic inflammation, via the MAS1 receptor, through suppression of ERK1/2- and NF-κB-dependent pathways. Br J Pharmacol. 2012 Jul;166(6):1964-76. doi: 10.1111/j.1476-5381.2012.04905.x.

15. Rodrigues-Machado MG1, Magalhães GS, Cardoso JA, Kangussu LM, Murari A, Caliari MV, Oliveira ML, Cara DC, Noviello ML, Marques FD, Pereira JM, Lautner RQ, Santos RA, Campagnole-Santos MJ. AVE 0991, a non-peptide mimic of angiotensin-(1–7) effects, attenuates pulmonary remodelling in a model of chronic asthma. Br J Pharmacol. 2013 Oct;170(4):835-46. doi: 10.1111/bph.12318.

16. Yoon HE, Kim EN, Kim MY, Lim JH, Jang IA, Ban TH, Shin SJ, Park CW, Chang YS, Choi BS. Age-Associated Changes in the Vascular Renin-Angiotensin System in Mice. Oxid Med Cell Longev. 2016;2016:6731093. doi: 10.1155/2016/6731093.
17. Wakahara S, Konoshita T, Mizuno S, Motomura M, Aoyama C, Makino Y, Kato N, Koni I, Miyamori I. Synergistic expression of angiotensin-converting enzyme (ACE) and ACE2 in human renal tissue and confounding effects of hypertension on the ACE to ACE2 ratio. Endocrinology. 2007 May;148(5):2453-7.

18. Agarwal D, Elks CM, Reed SD, Mariappan N, Majid DS, Francis J (2012). Chronic exercise preserves renal structure and hemodynamics in spontaneously hypertensive rats. Antioxid Redox Signal 16: 139–152.

19. Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, Diz DI, Gallagher PE. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. Circulation. 2005 May 24;111(20):2605-10.

20. Bolles M, Deming D, Long K, Agnihothram S, Whitmore A, Ferris M, Funkhouser W, Gralinski L, Totura A, Heise M, Baric RS. A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. J Virol. 2011 Dec;85(23):12201-15. doi: 10.1128/JVI.06048-11.

21. Huentelman MJ, Zubcevic J, Hernández Prada JA, Xiao X, Dimitrov DS, Raizada MK, Ostrov DA. Structure-based discovery of a novel angiotensin-converting enzyme 2 inhibitor. Hypertension. 2004 Dec;44(6):903-6.

22. Byrnes JJ1, Gross S, Ellard C, Connolly K, Donahue S, Picarella D. Effects of the ACE2 inhibitor GL1001 on acute dextran sodium sulfate-induced colitis in mice. Inflamm Res. 2009 Nov;58(11):819-27. doi: 10.1007/s00011-009-0053-3.

**Competing interests**
The author declares that he has no competing interests.

**Acknowledgements**
I have to thanks Genny del Zotto, Renato Zambello, Claudio Sorio, Nadir Mario Maraldi, Gregary Marhefka and Marco Artico for the support and encouragement

**Author Contributions**
LZ is sole author and sole investigator. The author conceived of the article and wrote it. LZ read and approved the final manuscript.