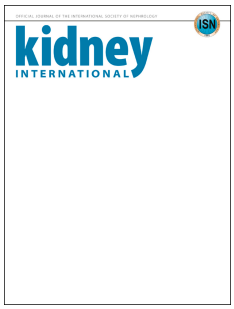

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COVID-19

Blockade of SARS-CoV-2 infection by recombinant soluble ACE2

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Viruses use specific host-cell plasma membrane molecules as receptors to enter the cell. SARS-CoV-2, the coronavirus responsible for the current COVID-19 pandemic, is believed to bind mainly or exclusively to angiotensin-converting enzyme 2 (ACE2), a cell surface protein otherwise known for its enzymatic, carboxypeptidase-type activity and its physiological role in the renin-angiotensin system (1,2). ACE2 activity per se does not seem to be involved in infectivity, but its role in pulmonary, cardiac and kidney function has putative consequences for disease severity.

ACE2, a friendly peptidase

ACE2 is a transmembrane protein with a large extracellular N-terminal domain bearing the active site. It was discovered through its structural homology with the dipeptidyl-peptidase angiotensin I-converting enzyme (kininase II, ACE). ACE2 removes the carboxyterminal amino-acid of angiotensin II (and also, but with reduced efficiency, of angiotensin I), inactivating angiotensin II and generating angiotensin 1-7 (3). Angiotensin 1-7, which activates a specific receptor, MAS is a vasodilator and has antioxidant and anti-inflammatory properties (4).

ACE2 is thus considered to have a counter-regulatory role, shifting to some extent the renin-angiotensin system from a vasoconstrictor to a vasodilator, endothelium-activator system, with favorable consequences for end-organ protection in experimental settings like ischemia or diabetes.

ACE2 and coronaviruses

Both SARS-CoV-2 and the coronavirus responsible for a more limited pandemic of respiratory syndromes seventeen years ago, SARS-CoV, use ACE2 for host cell entry and infection (1,2). Nonetheless, there does not seem to be strong evolutionary rationale for these viruses targeting ACE2 and the renin-angiotensin system, as another coronavirus involved in respiratory disease, MERS-CoV, does not bind ACE2 but dipeptidylpeptidase IV, another membrane-bound peptidase with different enzymatic specificity. Both peptidases are expressed in alveolar epithelial cells of the lung, although not in great abundance. In contrast, they are abundant in the kidney, especially in the proximal tubule. They are also present in podocytes. ACE2 is also expressed in the heart and is involved in myocardial

function (3). These organs might therefore be particularly prone to the effects of coronavirus infection.

ACE2 and treatment of COVID-19

To date, no specific treatment is available for COVID-19. The race is on amongst academic teams and pharmaceutical companies to identify effective agents and develop a vaccine. Besides strategies targeting the virus with antibodies to viral proteins or chemical coumpounds aimed at inhibiting viral replication, another approach is to focus on the cellular target of the virus, ACE2.

Binding of SARS-Cov-2 to ACE2 involves the viral Spike protein, which protrudes from the viral surface and is activated by human proteases. The molecular interaction between ACE2 and Spike has been modeled (1,2), and synthetic compounds or antibodies interfering in that interaction could be designed. Protease inhibitors preventing Spike activation could also be tested. Yet another therapeutic approach is the use of soluble ACE2 as a virus trap and inactivator. Soluble ACE2 generated by proteolytic cleavage of the membrane anchor is normally present in plasma, although at low concentration. Increasing its availability at tissue sites would shift the competition with membrane-bound ACE2 toward the soluble protein that cannot promote viral entry into the cell. It is also expected that this approach would preserve tissue ACE2 (5,6).

This sound theoretical rationale has now received experimental support from *in vitro* studies by Monteil et al (5) in SARS-CoV-2 infected monkey kidney-derived epithelial cells and engineered human blood vessel and kidney organoids. The authors isolated the SARS-CoV-2 from a nasopharyngeal sample of a patient with confirmed COVID-19 infection. After successful culture on Vero E6 cells (a line of African green monkey kidney cells), the isolated virus was sequenced by Next-Generation Sequencing and showed the prototypic coronal shape of viral particles by electron microscopy. The investigators then infected the Vero-E6 cells with SARS-CoV-2 and examined the effect of human recombinant soluble ACE2 (hrsACE2) added to the culture milieu. They found that exposure of infected cells to hrsACE2 during the first hour, followed by washing and incubation without hrsACE2, significantly inhibited SARS-CoV-2 replication 15 hours post infection. This inhibition was dependent on the initial quantity of the virus in the inoculum and the concentration of hrsACE2, establishing dose-dependency. In contrast to hsrACE-2, mouse recombinant soluble ACE2

did not inhibit the infection. Since the virus has a size of 80-100 nm, indicating that SARS-CoV-2 must first infect blood vessels prior to local tissue infections, the authors went on to infect human capillary organoids with the SARS-CoV-2 isolate. After demonstrating active viral replication in this experimental model, they found that the addition of hrsACE2 markedly reduced SARS-CoV-2 infection. Finally, since ACE2 is strongly expressed in tubular epithelium, the investigators examined the effect of hrsACE2 in kidney organoids generated from human embryonic stem cells. They first demonstrated ACE2 expression and active SARS-CoV-2 replication in organoids composed of proximal tubular cell and podocyte clusters. They then added hrsACE2 to the culture medium and found that it reduced SARS-CoV-2 infection in a dose dependent manner. What is still lacking is the demonstration that hrsACE2 can also protect pulmonary cells against SARS-CoV-2 infection. The **Figure** presents a schematic view of the renin-angiotensin system, emphasizing ACE2 with its two molecular forms, membranous and soluble, and their putative role in SARS-CoV2 cellular infection and virus trapping, respectively.

These observations are exciting in view of their potential clinical relevance. It remains to be seen whether hrsACE2 administration *in vivo* can overcome massive SARS-CoV-2 infection, considering also the stoichiometry of the association, with several Spike proteins per viral particle. There is some hope that this will be tested clinically, because hrsACE2 has been approved for human use and is currently being developed as treatment for acute respiratory distress syndromes (ARDS). A pharmacokinetic study in healthy volunteers and a phase II clinical trial in patients with ARDS have been completed (8,9).

ACE2 in other lung and kidney diseases

Studies have suggested that ACE2 protects the lung in experimental ARDS. This includes syndromes caused by chemical injury of the lung, bacterial infection, or infection by influenza virus or respiratory syncytial virus (see bibliography in reference 9). Genetic ACE2 deficiency worsens the condition in mice, while administration of recombinant ACE2 in mice or pigs improves experimental ARDS. Conversely, inactivation of ACE or angiotensin II AT1 receptor protects the lung. Genetic variation in ACE level has also been reported to be a prognostic factor for ARDS in humans. The balance between angiotensin II and angiotensin 1-7 may play a role in alveolar function in disease, with angiotensin II exerting deleterious and angiotensin 1-7 beneficial actions, but this remains to be further studied. In any case,

SARS-CoV infection and Spike activation result in ACE2 depletion, which might contribute to the severity of the respiratory disease in COVID-19 infection

ACE2 has also been shown to exert beneficial action in non-infectious experimental kidney disease, especially diabetic nephropathy, probably through both inactivation of angiotensin II and formation of angiotensin 1-7. Genetic deficiency of ACE2 in mice aggravates kidney pathology, and use of recombinant ACE2 has been advocated for treatment of angiotensin II-mediated kidney diseases (6). SARS-CoV-2 can infect the kidney (7), and the study by Monteil et al (5) shows that hrsACE2 dramatically reduces SARS-CoV-2 infection of human kidney organoids. In SARS-CoV-2 infection, ACE2 depletion might also aggravate pre-existing kidney disease.

The rationale for hrsACE2 therapy in COVID-19 is based primarily on its expected inhibitory effect on pulmonary infection, but it may have additional beneficial effects in the lung and other organs such as the kidney. It is noteworthy that sACE2 administration was well tolerated in clinical phase I and phase II trials in ARDS (8,9). Because ACE2 therapy is expected to result in angiotensin II depletion, attention should be paid to the impact on blood pressure and kidney function. Although additional studies are needed, the potentially protective action of hrsACE2 against viral infection gives new hope in the fight against COVID-19.

Figure legend

Schematic view of the renin-angiotensin system and the possible roles of SARS-CoV-2 binding to ACE2 in COVID-19. The physiological steps of the generation of angiotensin II and angiotensin (1-7) and their actions on specific receptors are shown in the left and middle part of the Figure. Angiotensin II is generated from angiotensinogen by the actions renin and subsequently ACE anchored in the cell membrane. ACE2, another transmembrane enzyme, removes the carboxyterminal amino-acid of angiotensin II, thereby inactivating angiotensin II but generating angiotensin 1-7 with biological activity distinct from angiotensin II. Angiotensin 1-7 activates the MAS receptor. On the right side, the interaction of circulating SARS-CoV-2 with ACE2 is depicted. Membrane bound ACE2 serves as the exclusive (or at least main) SARS-CoV-2 receptor, allowing host cell entry and infection via virus binding to the S1 domain of the viral Spike protein. In adddition to its full-length transmembranous form ACE2 also exists in soluble form (sACE2) in the circulation, lacking its membranous anchor. According to recent studies increasing bioavailability of sACE2 by administering recombinant human sACE2 may act as a virus trap and inactivator (5,6).

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