COVID–19 AND RAS BLOCKERS: A PHARMACOLOGY PERSPECTIVE

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During the spread of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) it has been noted that hypertension, coronary heart disease and diabetes, particularly in elderly people, increase susceptibility to SARS-CoV-2 infection.[1,2] Patients suffering from these diseases often taken blockers of the renin-angiotensin system, i.e. angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type 1 (AT$_1$) receptor blockers (ARBs). Since the receptor that allows coronavirus entry into cells is ACE2, it has been suggested that RAS blocker use per se is the reason for this increased susceptibility.[2] If true, this might imply that such drugs should no longer be used in COVID-19 patients. Yet, the resulting destabilizing of blood pressure control and lack of cardiovascular protection would carry major risks of precipitating strokes and heart attacks. Thus, how strong is the evidence supporting this concept, and should RAS blockers indeed be abandoned in such patients?

What is ACE2?

ACE2 is a "mono-carboxypeptidase", an enzyme capable of hydrolyzing a peptide bond at the carboxy-terminal (C-terminal) end of a protein or peptide. ACE2 received its name in 2000 because of its homology with ACE.[3] Yet, unlike ACE, ACE2 does not convert angiotensin I to angiotensin II, nor do ACE inhibitors block its activity. ACE2 hydrolyzes angiotensin II (=angiotensin-(1-8), a peptide consisting of 8 amino acids) to angiotensin-(1-7), and angiotensin I (=angiotensin-(1-10)) to angiotensin-(1-9) (Figure 1).[4] It additionally hydrolyzes multiple other peptides, including apelin, opioids, and kinins. Although both angiotensin-(1-7) and angiotensin-(1-9) are believed to have organ-protective properties that oppose those of angiotensin II, their normal in-vivo levels are often below detection limit. Most studies reporting protective effects therefore relied on the infusion of these peptides in rodents.

ACE2 is a membrane-bound enzyme.[4] Cleavage of its membrane-anchor ('shedding') by A Disintegrin And Metalloprotease 17 (ADAM17), also known as tumor necrosis factor α-converting enzyme (Figure 1), results in its appearance in body fluids in minute amounts. In pathological states, shedding of ACE2 is often increased, resulting in elevated soluble ACE2 levels in blood and urine.[5] Of interest, angiotensin II, by upregulating ADAM17, is among the factors that increase shedding.[6] However, the far majority of ACE2 is membrane-bound, and its soluble levels are very low, even under pathological conditions. Consequently, ACE2-mediated degradation of peptides depends on the membrane-bound version of the enzyme, not the soluble variant. This resembles fully what we know about ACE.[4]

ACE2 and the coronavirus

In addition to its role as a carboxypeptidase, ACE2 surprisingly turned out to be the receptor responsible for SARS coronavirus entry. Binding to ACE2 requires the surface unit of a viral spike protein (S1) (Figure 1),[7] while cell entry relies on 'priming' by the serine protease transmembrane protease, serine 2 (TMPRSS2).[7] Two recent reports confirmed that SARS-CoV-2 identically enters the cell.[8,9] SARS-CoV binding induces ACE2 downregulation.[10] In the lung, ACE2 expression occurs in type 2 pneumocytes and macrophages. Generally, however, pulmonary ACE2 expression is low when compared to other organs like the intestine, testis, heart and kidney.[11,12]

ACE2 and RAS blockers

ACE inhibitors are often confused with ACE2 inhibitors. Yet, they do not inhibit ACE2, and currently there are no drugs blocking ACE2 that can be applied to patients. Animal studies report that some ARBs upregulate ACE2 expression in the heart and the kidney.[13-16] Whether ACE inhibitors do the same is less certain.[13] Importantly, results were diverse, required high doses, and often differed per RAS blocker and per organ. No data have been reported for the lung, i.e., the most important site of virus entry. Confusingly, results at a high degree of RAS blockade (e.g., by applying 2 blockers at the same time) were found to be opposite to those observed at a lower degree of RAS blockade (by applying one RAS blocker).[13] The main reason for investigating ACE2 expression was the hope that high ACE2 levels would facilitate angiotensin-(1-7) formation, thereby increasing its protective effects in the heart or kidney.

Given the relationship between angiotensin II and ADAM17, a distinction should be made between membrane-bound and soluble ACE2. Theoretically, an increase in soluble ACE2 (implying more
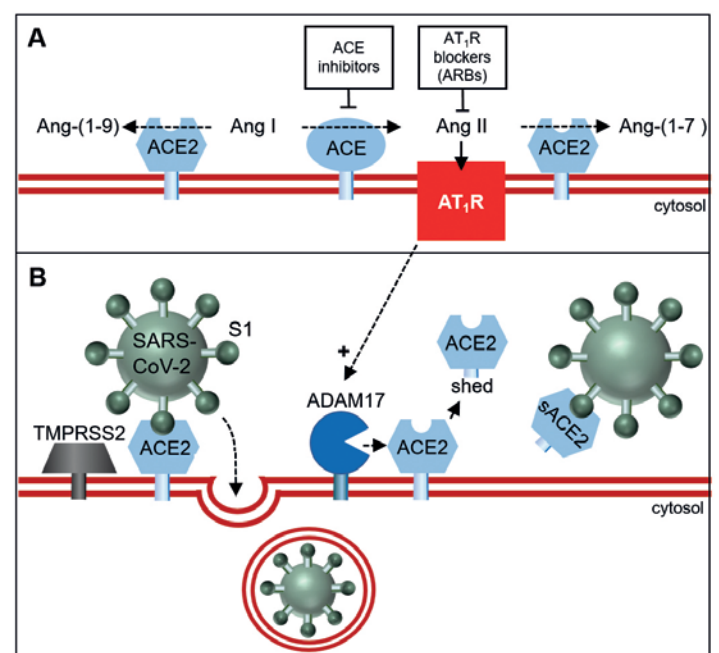

Figure 1. ACE2 degrades Ang I and II (A) and internalizes SARS-Cov-2 after TMPRSS2 priming (B). Shedding of membrane-bound ACE2 byADAM17 results in the release of soluble (s) ACE2, which might prevent virus entry by keeping it in solution. Ang II, via its type 1 receptor (AT1R), upregulates ADAM17. Derived from Danser et al..[16]

shedding) would result in less membrane-bound ACE2. Obviously, it is membrane-bound ACE2 that facilitates virus entry. Thus, when concluding that there is "upregulation" of ACE2, a crucial question is whether this concerns membrane-bound or soluble ACE2. Measuring membrane-bound ACE2 is technically challenging, and publications therefore often provide changes at the mRNA level only. In humans, data are usually limited to measurements of ACE2 activity in blood.[17] This reflects the soluble ACE2 protein circulating at very low levels. Whether this has any relevance for membrane-bound ACE2 is unknown, and possibly, as explained above, the relationship is inverse. If ARBs would upregulate membrane-bound ('tissue') ACE2, this is likely to be due to AT_1 receptor blockade. This implies that angiotensin II should have the opposite effect, i.e., it should downregulate membrane-bound ACE2. As discussed, angiotensin II-induced shedding is unlikely to underlie such downregulation. Acute studies in ACE2-transfected neuroblastoma cells have shown that angiotensin II additionally induces ACE2 internalization, thereby reducing the amount of ACE2 receptors on the cell surface.[18] Whether this also occurs in non-transfected (naturally ACE2-expressing) cells, chronically, and/or in vivo is unknown. Assuming there is a crucial role for AT_1 receptors, renin and ACE inhibitors should have the same directional effect as ARBs. Yet, for these drugs there is very limited (or none at all) data showing upregulation of ACE2.

Both ACE inhibitors and ARBs alter the levels of angiotensins: ACE inhibitors will increase angiotensin I, while ARBs increase both angiotensin I and II. It has been argued that increased levels of angiotensin II would impose an increased substrate load on the enzyme, thus requiring its upregulation.[19] Yet, the same might be argued for angiotensin I, since this is also hydrolyzed by ACE2. In reality, the carboxypeptidase ACE2 has multiple substrates, and an alteration in the level of one or two of these substrates (angiotensins I and II, occurring at fmol/mL levels, i.e., many orders of magnitude below the actual ACE2 concentration!) cannot possibly make a meaningful difference in its substrate load.

Taken together, there is evidence from animal studies that ARBs upregulate membrane-bound ACE2 whereas ACE inhibitors may not. The current data, however, are often conflicting and vary between ARBs and tissue (e.g., heart versus kidney). Even if the reported upregulation of tissue ACE2 by ARBs in animal studies could be extrapolated to humans, this would still not establish that it is sufficient to facilitate SARS-CoV-2 entry.

Beneficial effect of ACE2?

During acute lung injury alveolar ACE2 is downregulated.[10] This would decrease angiotensin II metabolism, thus resulting in higher local levels of this peptide. Since angiotensin II increases alveolar permeability and fosters lung injury, one might speculate that having increased ACE2 expression by preexisting ARBs treatment would actually be protective in the course of SARS-CoV-2 infection. Diminished myocardial ACE2 expression similarly facilitates inflammation and damage,[20] and low ACE2 levels predispose for hypertension and diabetes and their cardiovascular complications.[21,22] Given the multitude of ACE2 substrates, it is likely that its protective effects involve other substrates other than angiotensin II as well.

From this perspective, cardiovascular patients would be expected to display low ACE2 expression and thus be least susceptible to SARS-Cov-2 infection. This infection, if occurring, would rapidly deteriorate their situation by lowering ACE2 even further.[10] Simultaneously, RAS blockers, if exerting their effects in these patients via ACE2 upregulation, would be the most desirable drugs.[21,23] However, none of this is currently clinically proven.

What is needed now

Current data do not provide sufficient detail on variables of interest, like hypertension diagnosis and the antihypertensive drugs prescribed to test the hypotheses proposed. Appropriate controls are lacking, and corrections for age and other confounding factors should be applied. For instance, one cannot exclude that patients taking RAS blockers simply are older, display more hypertension and diabetes, and thus by definition are more susceptible to viral infection, independent of their use of RAS blockers. Additional questions are whether ACE2 quantity truly determines the degree of SARS-Cov-2 infection and/or relates to outcome, and, if such a relationship exists, to what degree RAS blockers affect it.

Conclusion

Clearly therefore, as advocated by all major cardiovascular societies in the world including the European Society of Hypertension (https://www.eshonline.org/spotlights/esh-stabtement-on-covid-19/), there is no reason to abandon or discontinue temporarily the use of RAS blockers preventatively in SARS-CoV-2 patients. Their proven therapeutic benefit outweighs any potential risk of them predisposing to corona infection. Moreover, it is unknown whether alternative antihypertensives do not carry the same 'risk'.

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