Effects of Renin-Angiotensin Inhibition on ACE2 (Angiotensin-Converting Enzyme 2) and TMPRSS2 (Transmembrane Protease Serine 2) Expression

Insights Into COVID-19

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ACE2 (angiotensin-converting enzyme 2) degrades Ang (angiotensin) I and II and is a cellular receptor for SARS-CoV-2, the virus that causes coronavirus disease 2019 (COVID-19). Viral entry into host cells occurs through binding of the viral spike (S) protein and ACE2. Preclinical data suggest that renin-angiotensin system (RAS) blockers upregulate ACE2. As a consequence, RAS blockers have been suggested to increase the risk of developing severe SARS-CoV-2 infection. However, recent large retrospective studies strongly argue against this hypothesis and rather suggest that RAS blockers be protective in such patients. Since the findings on RAS infection. However, recent large retrospective studies strongly argue against this hypothesis and rather suggest that RAS blockers be protective in such patients. Since the findings on RAS suppression, but rather reflects the nonspecific effects of a certain RAS blocker when applied at a high dose. Applying antisense oligonucleotides (ASO) as a tool to suppress the RAS would circumvent the latter. In the present study, we determined effects of an ACE inhibitor (enalapril) and an ARB (losartan) as well as AGT (angiotensinogen) ASO on tissue ACE2 in male C57BL/6J mice.

After 14 days of infusion, both enalapril and losartan increased plasma renin, measured as direct concentrations, by over 100-fold, confirming effective RAS inhibition (Figure [A]). ACE2 mRNA abundance was determined by quantitative PCR in lung, ileum, kidney, and heart tissues. Neither enalapril nor losartan changed the abundance of ACE2 mRNA in any of the tissues (Figure [B]). Ferrario et al reported that administration of lisinopril (an ACE inhibitor) or losartan for 12 days increases ACE2 mRNA abundance ≈3- to 5-fold in male rat heart. It is worth noting that ACE2 mRNA is much less abundant in heart compared with lung, ileum, and kidney (data not shown).

Viral entry also depends on TMPRSS2 (transmembrane protease serine 2) to prime S protein. TMPRSS2 mRNA was highly abundant in lung and ileum, moderately in kidney, while barely detectable in heart (data not shown). Thus, ACE2 and TMPRSS2 are co-expressed most abundantly in lung and ileum, consistent with their roles in SARS-CoV-2 infection. As with ACE2, TMPRSS2 mRNA abundance was not altered by either enalapril or losartan (Figure [E]).

Next, we determined whether depletion of AGT, the unique substrate of the RAS, changes ACE2 and TMPRSS2 mRNA abundance. AGT ASO led to elevation of plasma renin concentrations, an immediate consequence of RAS blockade (Figure [D]). However, abundance of ACE2 mRNA was unchanged in all tissues (Figure [E]). Interestingly, AGT ASO significantly decreased TMPRSS2 mRNA abundance in lungs (Figure [F]).

In summary, RAS inhibition did not affect mRNA abundance of ACE2 in male C57BL/6J mice administered enalapril, losartan, or AGT ASO. AGT ASO reduces TMPRSS2 mRNA expression in lungs, which is potentially protective against viral entry. These data support that RAS inhibition per se does not regulate ACE2 and hence is unlikely to increase the risk for COVID-19. In agreement with this concept, Sama et al recently were unable to detect changes in circulating ACE2 in patients taking RAS inhibitors.

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Disclosures

A. Daugherty and H.S. Lu filed a patent application for use of AGT (angiotensinogen) antisense oligonucleotides (ASO) in aortic aneurysmal disease. A.E. Mullick is an employee in Ionis Pharmaceuticals, Inc, who provided the AGT ASO. The other authors report no conflicts.

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**Figure.** Effects of enalapril, losartan, and AGT (angiotensinogen) antisense oligonucleotides (ASO) on ACE2 and TMPRSS2 (transmembrane protease serine 2) mRNA abundance. **A–C**, Male C57BL/6J mice were infused subcutaneously with either enalapril (3 mg/kg per day) or losartan (15 mg/kg per day) for 14 days. **D–F**, Male C57BL/6J mice were administered AGT ASO (80 mg/kg at day 1 and 4, 40 mg/kg at day 8 and 15) or vehicle (PBS) subcutaneously. Plasma renin (**A and D**) were measured by ELISA. **B, C, E, and F**, ACE2 and TMPRSS2 mRNA abundance were quantified by qPCR with TaqMan probes (ID: Mm01159003_m1 and ID: Mm00443687_m1, respectively) and normalized to the geometric mean of three reference genes: ACTB, GAPDH, and PPIA. Genes with a cycle threshold (Ct) >35 were considered undetectable. Error bars denote SEM; n=5/group. *P<0.001 vs vehicle, 1-way ANOVA with Holm-Sidak method. **P<0.05 vs vehicle, Student t test.