Effects of Renin-Angiotensin Inhibition on ACE2 and TMPRSS2 Expression

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Abstract

Angiotensin-converting enzyme 2 (ACE2), a component of the renin-angiotensin system, is a receptor for SARS-CoV-2, the virus that causes COVID-19. To determine whether the renin-angiotensin inhibition regulates ACE2 expression, either enalapril (an angiotensin-converting enzyme inhibitor) or losartan (an AT1 receptor blocker) was infused subcutaneously to male C57BL/6J mice for two weeks. Neither enalapril nor losartan changed abundance of ACE2 mRNA in lung, ileum, kidney, and heart. Viral entry also depends on transmembrane protease serine 2 (TMPRSS2) to prime the S protein. TMPRSS2 mRNA was abundant in lungs and ileum, modest in kidney, but barely detectable in heart. TMPRSS2 mRNA abundance was not altered by either enalapril or losartan in any of the 4 tissues. Next, we determined whether depletion of angiotensinogen (AGT), the unique substrate of the renin-angiotensin system, changes ACE2 and TMPRSS2 mRNA abundance. AGT antisense oligonucleotides (ASO) were injected subcutaneously to male C57BL/6J mice for 3 weeks. Abundance of ACE2 mRNA was unchanged in any of the 4 tissues, but TMPRSS2 mRNA was significantly decreased in lungs. Our data support that the renin-angiotensin inhibition does not regulate ACE2 and hence are not likely to increase risk for COVID-19.

Results and Discussion

Angiotensin-converting enzyme 2 (ACE2), a component of the renin-angiotensin system (RAS), is a cellular receptor for SARS-CoV-2, the virus that causes COVID-19. Viral entry into host cells occurs through binding of the viral spike (S) protein and ACE2.[1]

In the RAS, angiotensinogen (AGT) is cleaved by renin to produce angiotensin (Ang)I, which is then cleaved by angiotensin-converting enzyme (ACE) to generate AngII. AngII acts primarily through its type 1 receptors (AT1R) to regulate physiological and pathophysiological functions. Both AngI and AngII are substrates of ACE2, which antagonizes the pathophysiological consequences of ACE and AT1R. ACE inhibitors and AT1R blockers (ARBs) are widely used as the first-line drugs in patients with cardiovascular diseases. Because ACE, ACE2, and AT1R determine the generation and actions of AngII, there have been debates[2] whether patients treated with ACE inhibitors or ARBs have increased susceptibility to COVID-19. These debates have led to many retrospective studies published in recent weeks. Although these studies reported that COVID-19 either had no association with the use of either class of drugs or was more frequent in patients who are taking ACE inhibitors or ARBs, there are limited studies to determine whether ACE inhibitors or ARBs regulate ACE2 expression.

To determine whether RAS inhibition regulates ACE2 expression, either enalapril (an ACE inhibitor) or losartan (an ARB) was infused subcutaneously to male C57BL/6J mice via mini osmotic pumps. After 14 days of infusion, both enalapril and losartan increased plasma renin concentrations by over 100 folds, demonstrating effective RAS inhibition (**Figure A**). ACE2 mRNA abundance was determined by quantitative PCR (qPCR) in lung, ileum, kidney, and heart tissues. Neither enalapril nor losartan changed abundance of ACE2 mRNA in any of the tissues (**Figure B**). Ferrario et al.[3] reported that administration of lisinopril (an ACE inhibitor) or losartan for 12 days increases ACE2 mRNA abundance approximately 3 - 5 folds in male rat heart. It is worth noting that ACE2 mRNA is much less abundant in heart compared with lungs, ileum, and kidney (data not shown).

Viral entry also depends on transmembrane protease serine 2 (TMPRSS2) to prime S protein.[4] TMPRSS2 mRNA was highly abundant in lungs and ileum, moderately in kidney, while barely detectable in heart (data not shown). Thus, ACE2 and TMPRSS2 are co-expressed most abundantly in lungs and ileum, consistent with their roles in SARS-CoV-2 infection.[5] As with ACE2, TMPRSS2 mRNA abundance was not altered by either enalapril or losartan (**Figure C**).

Next, we determined whether depletion of AGT, the unique substrate of the RAS, changes ACE2 and TMPRSS2 mRNA abundance. AGT antisense oligonucleotides (ASO) were injected subcutaneously to male C57BL/6J mice. After 21 days of AGT depletion, plasma renin concentrations were elevated (**Figure D**), but 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, the RAS inhibition did not affect mRNA abundance of ACE2 in male C57BL/6J mice administered either an ACE inhibitor, ARB, or AGT ASO. AGT ASO reduces TMPRSS2 mRNA expression in lungs, which is potentially protective against viral entry. These data support that RAS inhibitors do not regulate ACE2 and hence are not likely to increase risk for COVID-19.

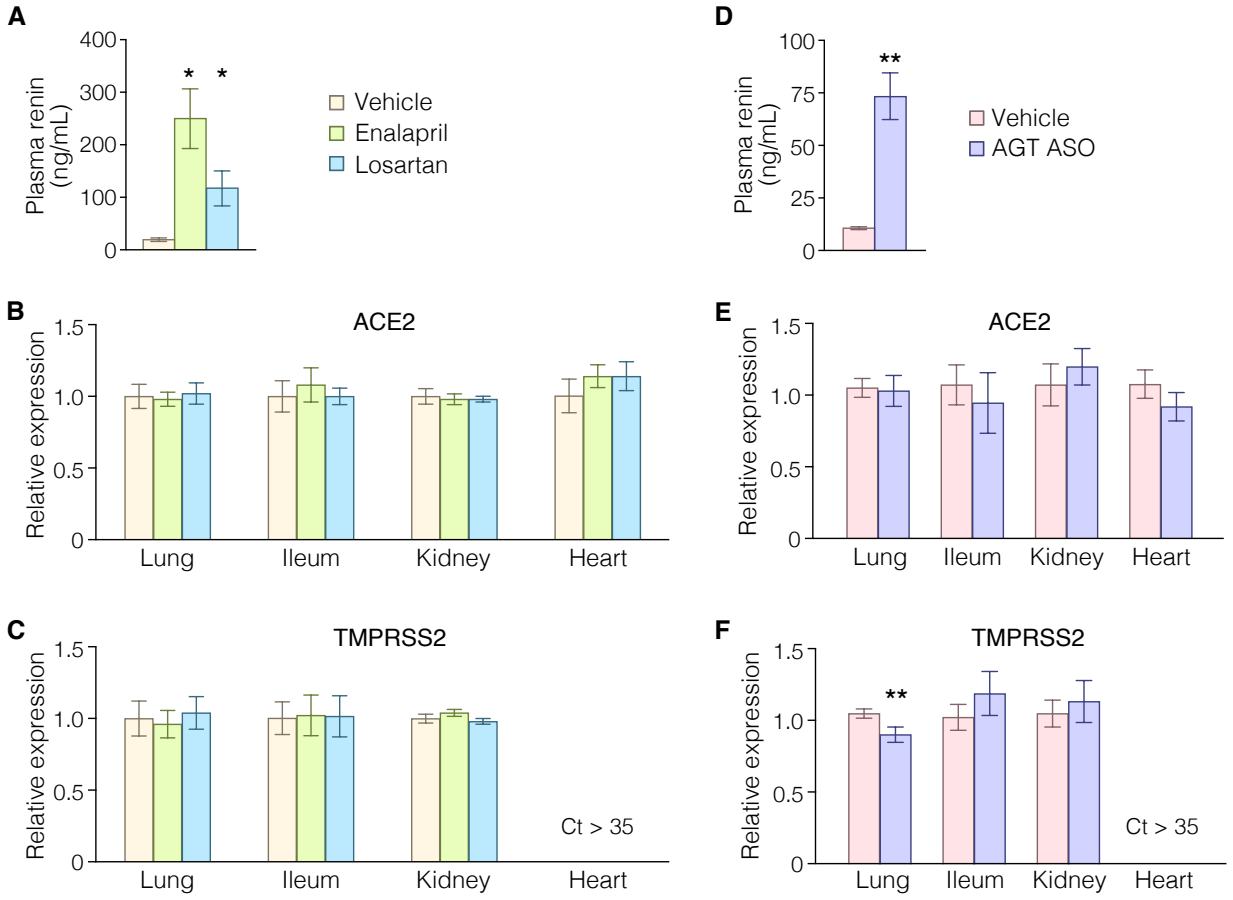

Figure. Effects of enalapril, losartan, and AGT ASO on ACE2 and TMPRSS2 mRNA abundance. **A-C**. Male C57BL/6J mice were infused subcutaneously with either enalapril (3 mg/kg/day) or losartan (15 mg/kg/day) for 14 days. **D-F**. Male C57BL/6J mice were administered AGT ASO (80 mg/kg at day 1, 4 and 40 mg/kg at day 8, 15) via subcutaneous injection. Plasma renin concentrations (**A** and **D**) were measured using an ELISA kit. (**B**, **C**, **E**, **F**) ACE2 and TMPRSS2 mRNA abundance were quantified by qPCR with TaqMan probes (ID: Mm01159003_m1 for ACE2 and ID: Mm00443687_m1 for TMPRSS2) and normalized to the geomean of three reference genes: ACTB, GAPDH, and PPIA. Genes with a threshold cycle (Ct) greater than 35 were considered undetectable. Error bars denote SEM; n = 5 for all experimental groups. * $P < 0.001$ versus vehicle, one-way ANOVA with Holm-Sidak method. ** $P < 0.05$ versus vehicle, Student's *t*-test.

Materials and Methods

The data that support the findings reported in this manuscript are available from the corresponding authors upon reasonable request.

Mini Osmotic Pump Implantation for Infusion of enalapril or losartan

Male C57BL/6J mice at the age of 8 weeks were implanted mini osmotic pumps (Alzet Model 2004; Durect Corp) subcutaneously to deliver water (control), enalapril (an ACE inhibitor; Sigma#E6888), or losartan (an ARB; Sigma#61188). Selected doses of enalapril 3 mg/kg/d and losartan 15 mg/kg/d were based on our previous study that demonstrated the selected doses reach maximal inhibition of AngII production and action, respectively.[6]

Administration of Antisense Oligonucleotides

Mouse AGT antisense oligonucleotides (AGT ASO) were provided by Ionis Pharmaceuticals Inc. On day 1 and day 4, PBS or AGT ASO 80 mg/kg was injected subcutaneously, followed by PBS or AGT ASO 40 mg/kg on day 8 and day 15. The selected doses of AGT ASO lead to the maximal inhibition of AGT mRNA, as determined by our previous study[7]. PBS was used as the control because ASOs were prepared and diluted in PBS.

Plasma Renin Concentrations

Plasma renin concentrations were measured using an ELISA kit (Mouse renin ELISA kit, R&D Systems#DY4277).

RNA Isolation and Quantitative PCR

Total RNA was extracted from tissues with an automated Maxwell RSC RNA extraction system (Promage#AS1340). qPCR reactions were performed with TaqMan Fast Advanced Master Mix (Thermo#4444556) and TaqMan probes (ID: Mm01159003_m1 for ACE2 and ID: Mm00443687_m1 for TMPRSS2) on QuantStudio 7. Data were analyzed using comparative Ct method and normalized by geometric mean of three reference genes: ACTB, GAPDH and PPIA.

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Disclosures

Alan Daugherty and Hong S. Lu have submitted a patent application for use of AGT ASO in aortic aneurysmal disease. Adam E. Mullick is an employee in Ionis Pharmaceuticals, Inc., who provided the AGT ASO. Ionis Pharmaceuticals did not provide funding for this study.

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