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RESEARCH ARTICLE

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A pressor dose of angiotensin II has no influence on the angiotensin-converting enzyme 2 and other molecules associated with SARS-CoV-2 infection in mice

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Abstract

In the early phase of the Coronavirus disease 2019 (COVID-19) pandemic, it was postulated that the renin-angiotensin-system inhibitors (RASi) increase the infection risk. This was primarily based on numerous reports, which stated that the RASi could increase the organ Angiotensin-converting enzyme 2 (ACE2), the receptor of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), in rodents. RASi can theoretically antagonize the potential influence of angiotensin II (Ang II) on ACE2. However, while Ang II decreases the ACE2 levels in cultured cells, there is little evidence that supports this phenomenon in living animals. In this study, we tested whether Ang II or Ang II combined with its antagonist would alter the ACE2 and other molecules associated with the infection of SARS-CoV-2. Male C57BL6/J mice were administered vehicle, Ang II (400 ng/kg/min), or Ang II with losartan (10 mg/kg/min) for 2 weeks. ACE2 knockout mice were used as a negative control for the ACE2 assay. We found that both Ang II, which elevated blood pressure by 30 mm Hg, and Ang II with losartan, had no effect on the expression or protein activity of ACE2 in the lung, left ventricle, kidney, and ileum. Likewise, these interventions had no effect on the expression of *Transmembrane Protease Serine 2* (*TMPRSS2*) and *Furin*, proteases that facilitate the virus-cell fusion, and the expression or activity of Tumor Necrosis Factor α-Convertase (TACE) that cleaves cell-surface ACE2. Collectively, physiological concentrations of Ang II do not modulate the molecules associated with SARS-CoV-2 infection. These results support the recent observational studies suggesting that the use of RASi is not a risk factor for COVID-19.

Abbreviations: ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; Ang II, angiotensin II; BP, blood pressure; COVID-19, coronavirus disease 2019; RAS, renin-angiotensin-system; RASi, renin-angiotensin-system inhibitors; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, Sprague-Dawley; TACE, tumor necrosis factor α-convertase; TMPRSS2, transmembrane protease serine 2.

Yu Wang and Hikari Takeshita are contributed equally to this work.

KEYWORDS
angiotensin II, angiotensin-converting enzyme 2, ARB, COVID-19, SARS-CoV-2

1 | INTRODUCTION

Angiotensin-converting enzyme (ACE) 2, named for its counter-regulatory role in the renin-angiotensin system (RAS), has been the target of diverse studies outside of the RAS owing to its multifunctional roles in many pathological and physiological conditions.[1] ACE2 is a zinc metalloprotease first reported in 2000 which shares homology with angiotensin-converting enzyme (ACE) in its catalytic domain and provides different key functions in the renin-angiotensin system (RAS).[2,3] Recently, the importance of ACE2 has gained new interest, as it is the functional receptor of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the new respiratory disease, Coronavirus disease 2019 (COVID-19), the pandemic that is currently ravaging through the world.[4]

In the early phase of the COVID-19 pandemic, an argument was put forth to understand if antihypertensive treatment using renin-angiotensin-system inhibitors (RASi), including ACE inhibitors and Angiotensin receptor blockades (ARBs), is associated with increased susceptibility or severity of COVID-19 in hypertensive patients.[5-7] This hypothesis is primarily based on the notion that RASi could increase the expression or activity of ACE2. Previous studies indicate that RASi increase ACE2 expression or activity in multiple organs, including the heart,[8-17] kidney,[8,12,13,18,19,20] aorta,[21-24] and lung,[25-27] in rodents. Theoretically, increased tissue ACE2 could increase the chances of virus invasion, resulting in the initiation of infection or local inflammation of each organ. However, recent observational studies indicate that the use of RASi is not associated with an increased risk of COVID-19 in patients with hypertension.[28-31] Therefore, it is desirable to find a theoretical basis to explain why the use of RASi might not increase the risk of COVID-19 despite its potential influence on ACE2. In our review, we raised several issues that need to be addressed in order to understand the actual impact of RASi on ACE2 in relation to SARS-CoV-2 infection. First, it is unclear how RASi increase tissue ACE2 in living animals. A simple interpretation of the in vivo phenomenon is conceivable that RASi antagonize the effects of angiotensin II (Ang II) on the transcriptional regulation of ACE2. It is reported that Ang II decreased the mRNA and protein levels of ACE2 in cultured rat astrocytes[32,33] and human kidney epithelial cells.[34] However, the concentration of Ang II used in these studies (10^{-7}-10^{-6} M) was far higher than the physiological levels in the plasma of mice ($<1 \times 10^{-11}$ M),[35] rats ($<1 \times 10^{-10}$ M),[36] and humans ($<1\text{-}2 \times 10^{-11}$ M).[37] Interestingly, Petel et al reported that a 2-week infusion of Ang II in mice increased the mRNA expression of ACE2 in the heart, but paradoxically decreased the cardiac protein levels of ACE2. This was accompanied by the increased activity of the tumor necrosis factor-α conversion (TACE) that decreases the levels of cell surface full-length ACE2 by ectodomain shedding.[38] However, it should be noted that the concentration of Ang II (1.5 mg/kg/day) used in the study was high enough to increase BP by approximately 1.5-fold, and plasma Ang II concentration around 15-fold.[38] In contrast to the positive influence of Ang II on the mRNA expression of ACE2 reported by Petel et al.[38] Sriramula et al reported that Ang II infusion at 200 ng/kg/min for 2 weeks reduced mRNA and protein expression of ACE2 in the periventricular nucleus accompanied by 1.6-fold increase of BP in male Sprague-Dawley (SD) rats.[39] Gonzalez et al also reported that Ang II infusion at 400 ng/kg/min for 2 weeks reduced protein expression of ACE2 in the renal cortex and medulla accompanied by an increase of systolic BP up to over 200 mm Hg in male SD rats.[40] Collectively, Ang II at a level that could induce a vigorous increase in BP appears to decrease protein expression or activity of tissue ACE2 with the inconsistent transcriptional effect on this gene in rodents. Nevertheless, it remains to be understood whether the influence of Ang II on ACE2 is biologically relevant in animals at concentrations that could mimic physiological conditions. Second, the cellular invasion of SARS-CoV-2 depends not only on ACE2 but also on proteases, including Transmembrane Protease Serine 2 (TMPRSS2) and Furin. Spike proteins of SARS-CoV-2 bind to the cell surface ACE2, followed by proteolytic cleavage by these proteases, which results in the endocytosis of the virus.[41] It was recently reported that an ARB, losartan, or an ACE inhibitor, enalapril did not affect the organ expression of ACE2 and TMPRSS2 in male C57BL/6J mice.[42] However, it remains unknown whether Ang II or Ang II in combination with RASi would affect the expression of these proteases. Third, the effect of Ang II or RASi on the expression or activity of TACE, which is also a determinant of the localization of ACE2 on the cell surface, needs to be examined to understand the net influence of RASi on SARS-CoV-2 infection. Finally, as most studies analyzed the effect of RASi in each organ individually, there is little knowledge about the relative effects of RASi on ACE2 in different organs.

To address these issues, we conducted a cross-organ analysis of the lung, small intestine, heart, and kidney in mice infused with a lower dose of Ang II than previously reported,[38] to understand the effect of Ang II or ARB on ACE2 and the associated molecules involved in the tissue invasion of SARS-CoV-2. Samples from ACE2 knockout mice were used as negative controls to confirm the assay specificity of ACE2 in the present study.

2 | METHODS

2.1 | Experimental animals and protocols

Wild-type C57BL/6 mice were purchased from CLEA Japan. ACE2 knockout (ACE2KO) mice with a C57/BL6 background were generated as previously described.[43] Mice were fed standard chow and water ad libitum. A schematic of the study protocol is shown in Figure 1A. At 14 to 15 week of age, male wild-type mice were randomly assigned to three groups, and they received either Ang II (400 ng/kg/min) (n = 9) or Ang II with losartan (10 mg/kg/min) (n = 8) or saline (n = 8) for 2 weeks via subcutaneous implantation of osmotic mini-pumps (model 1004, ALZET Corp, Palo Alto, CA). The concentration of losartan was determined based on previous papers.[44-46] After 2 weeks of infusion, tissues were excised, weighed, and subjected to further analysis. Blood pressure (BP) was measured before and after the administration of reagents using the tail-cuff method (Softron Co, Tokyo, Japan). Three 19-week-old and one 27-week-old ACE2KO mice were used as a negative control for the evaluation of ACE2 gene expression and activity, and tissues were collected in the same manner as that for wild-type mice. During the procedure of the implantation of osmotic mini-pumps and tissue collection, mice were anesthetized with intraperitoneal injection of a mixture of medetomidine (0.3 mg/kg), midazolam (4 mg/kg), and butorphanol (5 mg/kg) following the guideline of Osaka University Institutional Animal Care and committee.

The study protocol was approved by the Osaka University Institutional Animal Care and Use Committee and was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2 | Collection of tissue samples

The left ventricle, lung, kidney, and ileum were collected, weighed, and divided into three parts for RNA extraction, and the evaluation of ACE2 and TACE protein activity. For RNA extraction, tissues were stored in RNAlater according to the manufacturer's instructions (Thermo Fisher Scientific, USA). For the activity evaluation, tissues were immediately frozen in liquid nitrogen and stored at −80°C until further use.

2.3 | ACE2 activity assay

ACE2 activity was measured using an MCA-based peptide substrate in the ACE2 assay kit (ab273297, Abcam, UK) according to the manufacturer's instructions with the modification based on previous reports.[38,47] Briefly, tissues were homogenized in the assay buffer containing a protease inhibitor cocktail (78415, Thermo Fisher Scientific, USA), using TissueLyser LT (Qiagen, Germany). Thereafter, tissue homogenates were incubated with protease inhibitors, including 10 μmol/L of captopril (07134-21, Nacalai Tesque, Japan), 5 μmol/L of amastatin (A1276, Sigma-Aldrich, USA), 10 μmol/L of bestatin (B8385, Sigma-Aldrich, USA), and 10 μmol/L of Z-Pro-prolinal (BML-PI112-0005, Enzo Life Sciences, USA) in the absence or presence of 10μmol/l of MLN-4760 (CS-0015513, Chemscene, USA), an ACE2 inhibitor at 37°C for 30min. Fluorescence intensity was monitored every 5 min for 30 min and measured using a Tecan SPARK multimode microplate reader with appropriate filters (Ex/Em = 320/420 nm) (Tecan Trading AG, Switzerland). The protein concentration of the homogenate was

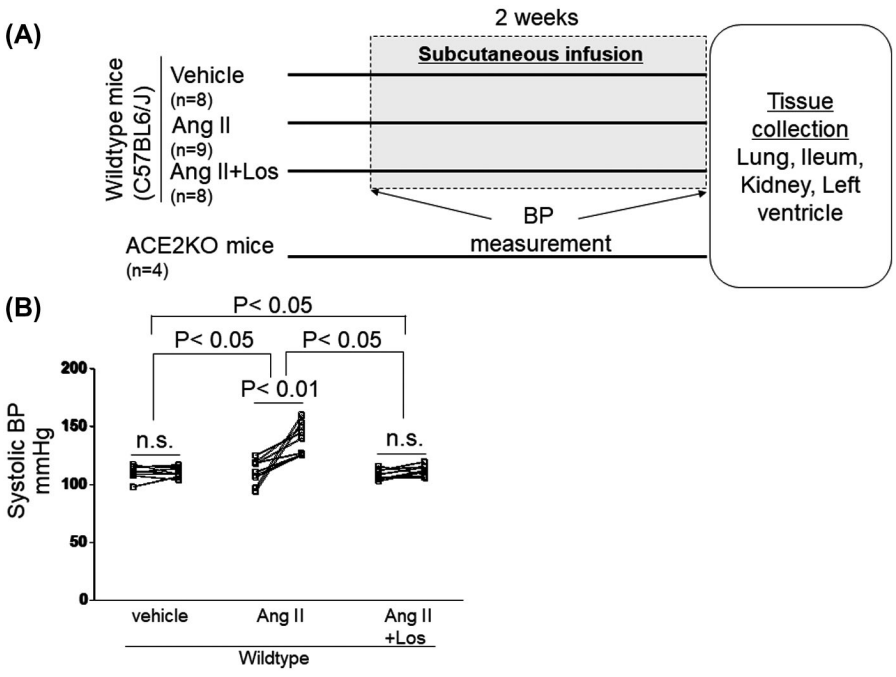

FIGURE 1 Schematic overview and the influence of Ang II infusion on blood pressure (BP). A, Schematic overview. B, BP before and after the infusion of Ang II (400 ng/kg/min), Ang II with losartan (10 mg/kg/min) or saline. Difference between BP before and after the infusion in each group was analyzed with paired *t* test. Difference in the before-after change in BP among the groups was analyzed by repeated measures ANOVA with the Tukey post hoc test. Ang II, angiotensin II; Los, losartan

determined with the Bradford protein assay (Bio-Rad Protein Assay Reagent, Bio-Rad Laboratories). Tissue ACE2 activity was defined as the amount of enzyme that catalyzes the release of 1 pmol of MCA per min per mg protein from the substrate under the assay conditions at 25°C (pmol/min/mg protein). The final ACE2 activity was determined by subtracting the measurement value in the presence of MLN-4760 from that in the absence of MLN-4760.

2.4 | TACE activity assay

TACE activity was assessed using the SensoLyte 520 TACE activity assay (AnaSpec, San Jose, CA) kit according to the manufacturer's instructions. Tissues were homogenized in the assay buffer using TissueLyser LT (Qiagen, Germany). Fluorescence intensity was monitored every 5 min for 15 min and measured using a Tecan SPARK multimode microplate reader with filters to detect the fluorescence (Ex/Em = 490/520 nm). (Tecan Trading AG, Switzerland). The protein concentration of the homogenate was determined using the Bradford protein assay (Bio-Rad Protein Assay Reagent, Bio-Rad Laboratories). Tissue TACE activity was calculated to represent the amount of enzyme that catalyzes the release of 1 μmol of 5-FAM per min per mg protein from the substrate under the assay conditions at 25°C (μmol/min/mg protein).

2.5 | RNA extraction and quantitative RT-PCR-based gene expression analysis

Total RNA from the lung, ileum, and kidney was isolated using Sepasol (R)-RNA Super G (09379-55; Nacalai Tesque, Japan) after treatment with DNase (Code No. 2270A, Takara Bio Company, Japan). Total RNA of the left ventricle was isolated using the ReliaPrepTM RNA Tissue Miniprep System (Z6110, Promega, USA). cDNA synthesis was performed with a ReverTra Ace qPCR RT kit (FSQ-101, Toyobo, Japan) according to the manufacturer's instructions. All genes were evaluated using the SYBR green qPCR system (Applied Biosystems 7900HT Fast real-time PCR system, Thermo Fisher Scientific, USA) with the primers listed in Table S1. Relative expression was calculated using the ΔΔCt method with normalization to the *18S* gene.

2.6 | Statistical analysis

The minimal sample size in each group was calculated as 7 by Power and Sample Size Calculation 3.1 to detect a 50% difference in the real-time PCR analysis, with a standard deviation of 0.3 when the expression level of vehicle treatment was determined as 1 (α error = 0.05, β error = 0.2). All data are presented as mean ± SEM. Difference in BP before and after the administration of Ang II, Ang II with losartan, or saline was analyzed by paired *t* test, and difference in the before-after change in BP among the groups was analyzed by repeated measures ANOVA. Differences among multiple groups were analyzed by one-way ANOVA with Bonferroni testing. An outlier in each group in each analysis was excluded when determining significance by Grubbs' test with α error < 0.01.

3 | RESULTS

3.1 | Effects of Ang II or ARB on BP

Two-week continuous infusion of Ang II at 400 ng/kg/min increased the systolic BP by approximately 30 mm Hg (before to after the infusion, 111 ± 4 mm Hg to 139 ± 5 mm Hg, respectively, $P < .01$) (Figure 1B). Co-administration of losartan blunted the pressor effect of Ang II (109 ± 2 mm Hg to 112 ± 2 mm Hg, $P = .15$) (Figure 1B). There were no differences in the body weight and organ weight of the lung, ileum, kidney, and left ventricle among the groups (Table S2).

3.2 | Effect of Ang II or ARB on the tissue expression of *ACE2*, *TMPRSS2*, *Furin,* and *TACE*

The expression of *ACE2* and the associated genes involved in the tissue invasion of SARS-CoV-2 was analyzed using real-time PCR in the lung, ileum, kidney, and left ventricle. We confirmed that the expression of *ACE2* was the highest in the ileum and the lowest in the left ventricle (Figure 2A). In all the organs tested, neither Ang II nor Ang II with losartan altered the expression of *ACE2*, which was absent in ACE2 knockout mice (Figure 2B). Consistent with previous reports, the expression of *TMPRSS2* was very low in the left ventricle (Figure 2A).[48] We did not find any difference in the expression of *TMPRSS2, Furin,* and *TACE* in response to Ang II or Ang II with losartan in the tested organs (Figure 2B).

3.3 | Effects of Ang II or ARB on tissue activity of ACE2 and TACE

We measured tissue ACE2 activity using the conventional MCA-based peptide substrate under the treatment with several protease inhibitors including an ACE inhibitor, captopril.[38,47,49] In all the organs, neither Ang II nor Ang II with losartan altered the ACE2 activity in wild-type mice (Figure 3A). In addition, the TACE activity was also not altered by Ang II or Ang II with losartan in the lung, ileum, kidney, and left ventricle (Figure 3B).

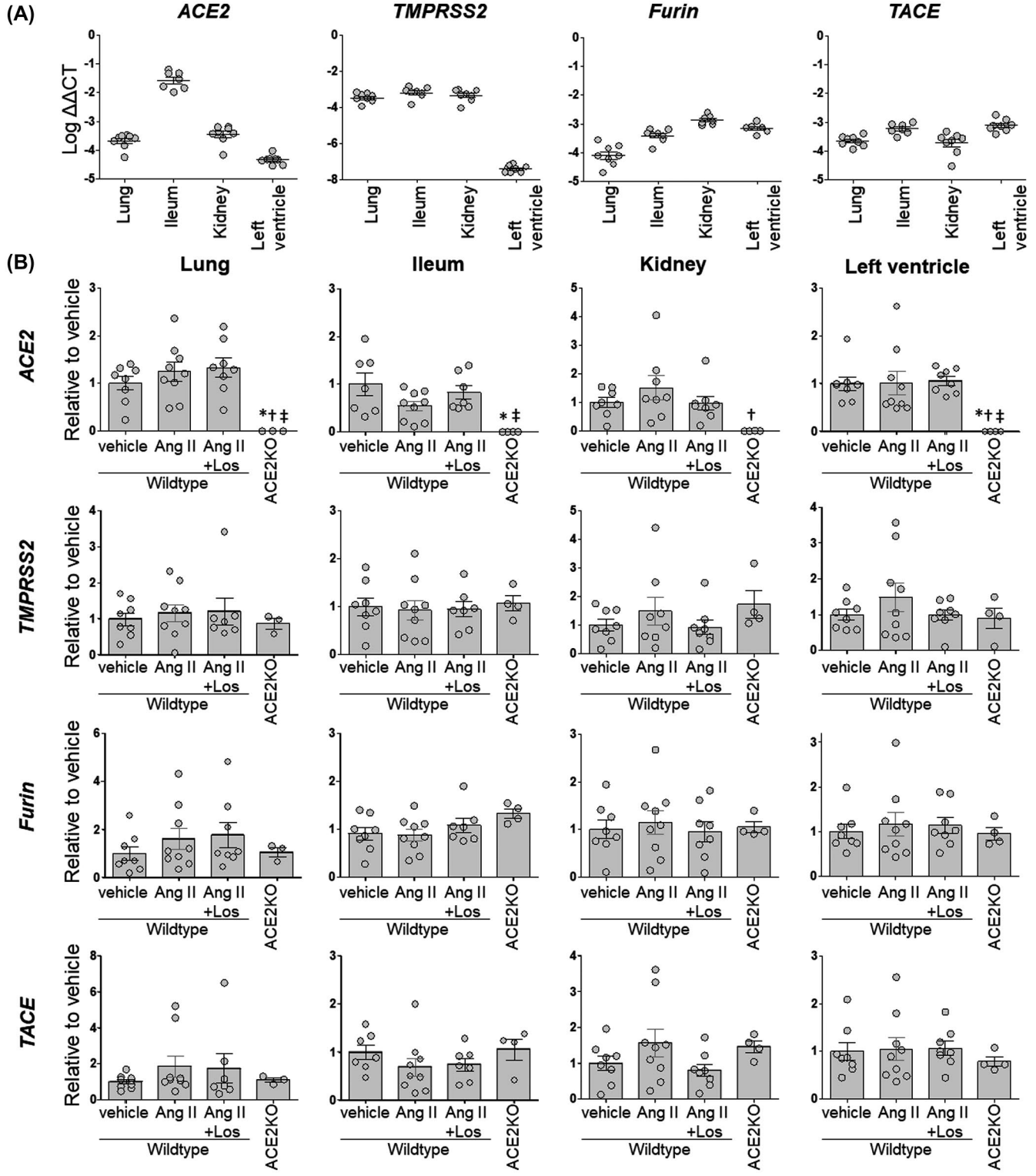

FIGURE 2 RT-PCR analysis of tissue expression of *ACE2, TMPRSS2, Furin*, and *TACE* in mice. A, Comparison of the expression of the corresponding genes across the organs in vehicle-treated wild-type mice. Y-axis represents log-transformed ΔΔCt of each gene normalized to *18S*. B, The expression of the corresponding genes among wild-type mice with Ang II (400 ng/kg/min), Ang II with losartan (10 mg/kg/min) or saline, and ACE2KO mice. Differences were analyzed with a one-way ANOVA using the Bonferroni post hoc test. $^{*}P < 0.05$ vs. wild-type mice with saline. $^{\dagger}P < 0.05$ vs. wild-type mice with Ang II. $^{\ddagger}P < 0.05$ vs. wild-type mice with Ang II and losartan

4 | DISCUSSION

In the current study, we found that a 2-week administration of Ang II increased the systolic BP by approximately 30 mm Hg. However, this did not alter the mRNA abundance of *ACE2* in multiple organs, including the lung and ileum, which are the vulnerable sites for the initial invasion of SARS-CoV-2. The same was the case for the left ventricle and kidney, where local inflammation by direct viral invasion is associated with the severity of COVID-19.[50-54] Ang II also did not alter the expression of *TMPRSS2* and *Furin,* proteases that facilitate viral invasion after binding to ACE2, and *TACE*, which regulates the surface expression of ACE2 by proteolytic cleavage. Losartan, an ARB that antagonized the pressor effect of Ang II, did not affect the tissue expression of *ACE2* and the associated genes in all the tissues tested. In accordance with the transcript analysis, we found that neither Ang II nor Ang II with losartan had an effect on the tissue activity of ACE2 and TACE. Collectively, the current findings clearly excluded the possibility that a pressor dose of Ang II is a potent regulator of ACE2 and the genes associated with the SARS-CoV-2 infection in rodents (Figure 4). Accordingly, ARBs did not appear to alter these molecules by antagonizing the effect of Ang II. Considering the numerous reports on the effects of RASi on the tissue levels of ACE2, it is safe to assume that factors other than the increase in Ang II are involved in the effect of these drugs on ACE2 expression.

High doses of Ang II downregulate ACE2 in cultured cells.[32-34] Koka et al reported that Ang II at 0.5 μM, but not 0.25 μM, decreased the expression of *ACE2* in cultured human kidney tubular cells.[34] This suggests that Ang II downregulates *ACE2* not simply by provoking specific downstream cellular signaling but by boosting vigorous cellular inflammation. Conversely, it is assumed that RASi could normalize the expression of *ACE2*, which is reduced because of cellular inflammation. This hypothesis is in line with previous reports where *ACE2* was reduced by experimental intervention to provoke tissue injury in rodents, including experimental autoimmune myocarditis,[9] experimental CHF induced by an aortocaval fistula,[14] subtotal nephrectomy,[20] myocardial infarction by ligation of the left coronary artery,[11] and pulmonary injury induced by lipopolysaccharide[26,27] or cigarettes.[25] RASi restored the expression or activity of ACE2 in these experimental models, accompanied by the alleviation of the pathological condition.[9,11,14,20,25-27] In addition, the hypothesis that the effect of RASi on *ACE2* depends on their ability to attenuate tissue inflammation is also consistent with a previous report that high concentrations of Ang II decreased cardiac ACE2 protein levels despite the paradoxical increase in transcript levels as a result of increased TACE activity, which is commonly activated by inflammatory stimuli.[38,55] Therefore, the potential effect of RASi on tissue *ACE2* can be attributed to their ability to alleviate organ inflammation

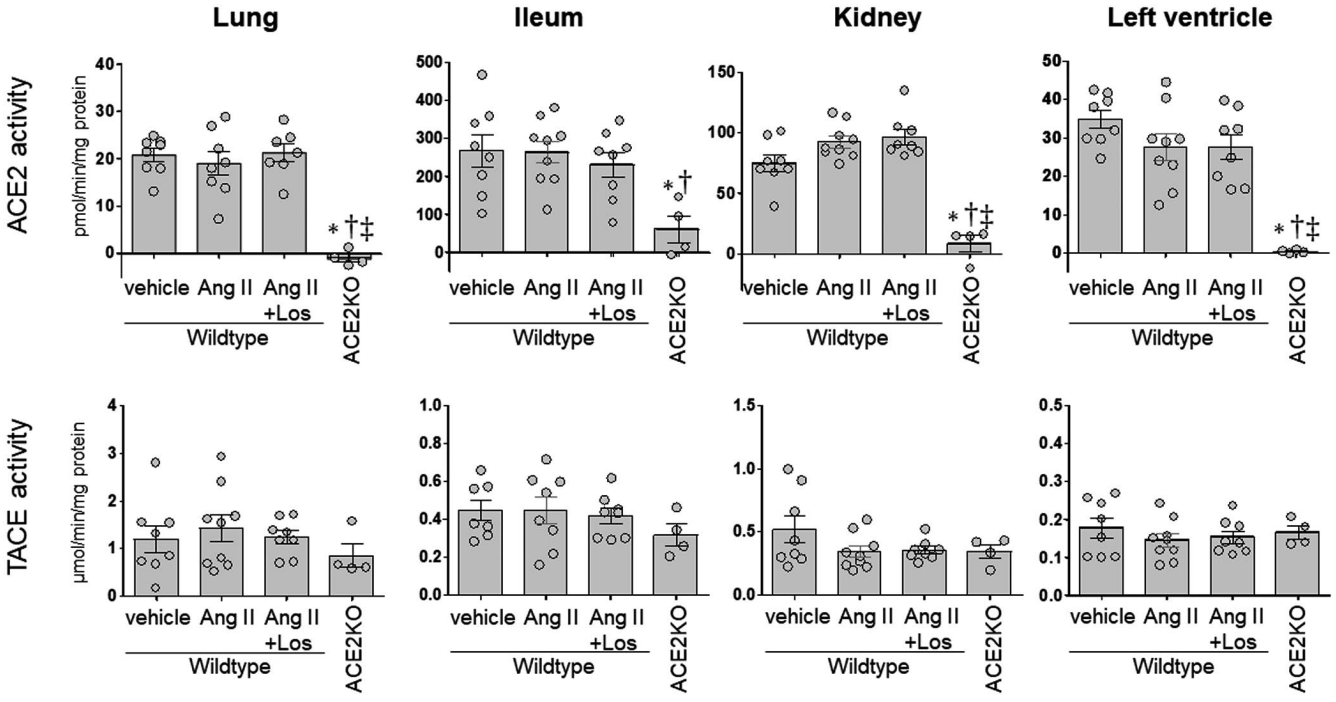

FIGURE 3 Tissue activity of ACE2 and TACE in mice. The activity of (A) ACE2 and (B) TACE among wild-type mice with Ang II (400 ng/kg/min), Ang II with losartan (10 mg/kg/min) or saline, and ACE2KO mice. Differences were analyzed with a one-way ANOVA using the Bonferroni post hoc test. *$P < .05$ vs. wild-type mice with saline. †$P < 0.05$ vs. wild-type mice with Ang II. ‡$P < .05$ vs. wild-type mice with Ang II and losartan

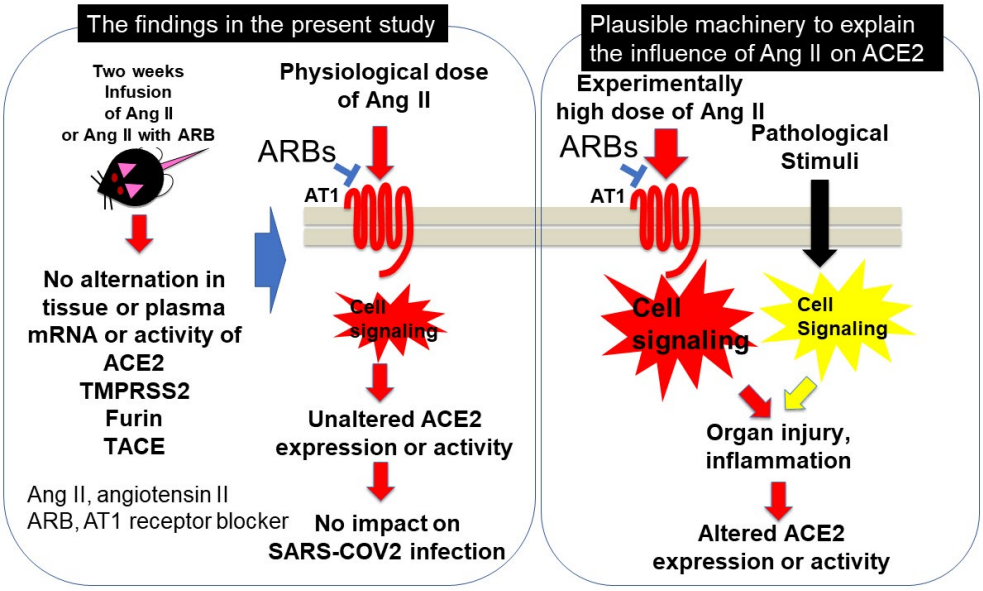

FIGURE 4 Graphical abstract

and damage (graphical abstract in Figure 4). This is consistent with recent observational studies suggesting that the use of RASi is not a risk in the treatment of COVID-19.[28-30]

As we used 15-weeks-old young male mice in the study, our findings should be interpreted cautiously when considering the effect of angiotensin II or RASi on female or aged animals. Particularly, aging is considered a great risk for COVID-19 and the effect of angiotensin II on ACE2 and associated molecules might be different depending on the age of animals.

In summary, we found that the 2-week infusion of Ang II in normal mice increased the BP but did not alter the expression or activity of ACE2 and other molecules associated with SARS-CoV-2 infection. Accordingly, the inhibition of Ang II by ARB had no influence on the expression or activity of these molecules. This study clearly indicated that Ang II is not a direct transcriptional regulator of *ACE2* in vivo, and the effect of RASi on *ACE2* might depend on their ability to repair organ injury. Therefore, this study could provide a theoretical basis for the fact that the use of RASi is not a risk in hypertensive patients in this COVID-19 era.

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CONFLICT OF INTEREST

The authors have no financial conflicts of interest.

AUTHOR CONTRIBUTIONS

Y. Wang and H. Takeshita designed and performed experiments and assisted in writing the manuscript. K. Yamamoto conceived the study, designed experiments, and wrote the manuscript. Y. Huang, C. Wang, T. Nakajima, Y. Nozato, T. Fujimoto, S. Yokoyama, K. Hongyo, F. Nakagami, H. Akasaka, Y. Taka, Y. Takeya, and Ken Sugimoto performed experiments. H. Rakugi designed experiments.

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REFERENCES

1. Gheblawi M, Wang K, Viveiros A, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. *Circ Res*. 2020;126:1456-1474.
2. Donoghue M, Hsieh F, Baronas E, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circ Res*. 2000;87:E1-E9.
3. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem*. 2000;275:33238-33243.
4. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579:270-273.
5. Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD. Renin-angiotensin-aldosterone system inhibitors in patients with Covid-19. *N Engl J Med*. 2020;382:1653-1659.
6. Danser AHJ, Epstein M, Batlle D. Renin-angiotensin system blockers and the COVID-19 pandemic: at present there is no evidence to abandon renin-angiotensin system blockers. *Hypertension*. 2020;75:1382-1385.
7. Brown JD. Antihypertensive drugs and risk of COVID-19? *Lancet Respir Med*. 2020;8:e28.

8. Iwanami J, Mogi M, Tsukuda K, et al. Role of angiotensin-converting enzyme 2/angiotensin-(1–7)/Mas axis in the hypotensive effect of azilsartan. *Hypertens Res*. 2014;37:616-620.
9. Sukumaran V, Veeraveedu PT, Gurusamy N, et al. Cardioprotective effects of telmisartan against heart failure in rats induced by experimental autoimmune myocarditis through the modulation of angiotensin-converting enzyme-2/angiotensin 1–7/mas receptor axis. *Int J Biol Sci*. 2011;7:1077-1092.
10. Takeda Y, Zhu A, Yoneda T, Usukura M, Takata H, Yamagishi M. Effects of aldosterone and angiotensin II receptor blockade on cardiac angiotensinogen and angiotensin-converting enzyme 2 expression in Dahl salt-sensitive hypertensive rats. *Am J Hypertens*. 2007;20:1119-1124.
11. Ocaranza MP, Godoy I, Jalil JE, et al. Enalapril attenuates downregulation of Angiotensin-converting enzyme 2 in the late phase of ventricular dysfunction in myocardial infarcted rat. *Hypertension*. 2006;48:572-578.
12. Jessup JA, Gallagher PE, Averill DB, et al. Effect of angiotensin II blockade on a new congenic model of hypertension derived from transgenic Ren-2 rats. *Am J Physiol Heart Circ Physiol*. 2006;291:H2166-2172.
13. Agata J, Ura N, Yoshida H, et al. Olmesartan is an angiotensin II receptor blocker with an inhibitory effect on angiotensin-converting enzyme. *Hypertens Res*. 2006;29:865-874.
14. Karram T, Abbasi A, Keidar S, et al. Effects of spironolactone and eprosartan on cardiac remodeling and angiotensin-converting enzyme isoforms in rats with experimental heart failure. *Am J Physiol Heart Circ Physiol*. 2005;289:H1351-H1358.
15. Ferrario CM, Jessup J, Chappell MC, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation*. 2005;111:2605-2610.
16. Burrell LM, Risvanis J, Kubota E, et al. Myocardial infarction increases ACE2 expression in rat and humans. *Eur Heart J*. 2005;26:369-375; discussion 322–364.
17. Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. *Hypertension*. 2004;43:970-976.
18. Ferrario CM, Jessup J, Gallagher PE, et al. Effects of renin-angiotensin system blockade on renal angiotensin-(1–7) forming enzymes and receptors. *Kidney Int*. 2005;68:2189-2196.
19. Soler MJ, Ye M, Wysocki J, William J, Lloveras J, Batlle D. Localization of ACE2 in the renal vasculature: amplification by angiotensin II type 1 receptor blockade using telmisartan. *Am J Physiol Renal Physiol*. 2009;296:F398-F405.
20. Velkoska E, Dean RG, Burchill L, Levidiotis V, Burrell LM. Reduction in renal ACE2 expression in subtotal nephrectomy in rats is ameliorated with ACE inhibition. *Clin Sci (Lond)*. 2010;118:269-279.
21. Zhang YH, Hao QQ, Wang XY, et al. ACE2 activity was increased in atherosclerotic plaque by losartan: possible relation to anti-atherosclerosis. *J Renin Angiotensin Aldosterone Syst*. 2015;16:292-300.
22. Jin HY, Song B, Oudit GY, et al. ACE2 deficiency enhances angiotensin II-mediated aortic profilin-1 expression, inflammation and peroxynitrite production. *PLoS One*. 2012;7:e38502.
23. Zhong JC, Ye JY, Jin HY, et al. Telmisartan attenuates aortic hypertrophy in hypertensive rats by the modulation of ACE2 and profilin-1 expression. *Regul Pept*. 2011;166:90-97.
24. Igase M, Strawn WB, Gallagher PE, Geary RL, Ferrario CM. Angiotensin II AT1 receptors regulate ACE2 and angiotensin-(1–7) expression in the aorta of spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol*. 2005;289:H1013-H1019.
25. Han SX, He GM, Wang T, et al. Losartan attenuates chronic cigarette smoke exposure-induced pulmonary arterial hypertension in rats: possible involvement of angiotensin-converting enzyme-2. *Toxicol Appl Pharmacol*. 2010;245:100-107.
26. Li Y, Zeng Z, Li Y, et al. Angiotensin-converting enzyme inhibition attenuates lipopolysaccharide-induced lung injury by regulating the balance between angiotensin-converting enzyme and angiotensin-converting enzyme 2 and inhibiting mitogen-activated protein kinase activation. *Shock*. 2015;43:395-404.
27. Wosten-van Asperen RM, Lutter R, Specht PA, et al. Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1–7) or an angiotensin II receptor antagonist. *J Pathol*. 2011;225:618-627.
28. Mancia G, Rea F, Ludergnani M, Apolone G, Corrao G. Renin-angiotensin-aldosterone system blockers and the risk of Covid-19. *N Engl J Med*. 2020;382:2431-2440.
29. Gao C, Cai Y, Zhang K, et al. Association of hypertension and antihypertensive treatment with COVID-19 mortality: a retrospective observational study. *Eur Heart J*. 2020;41:2058-2066.
30. Fosbol EL, Butt JH, Ostergaard L, et al. Association of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker use with COVID-19 diagnosis and mortality. *JAMA*. 2020;324(2):168.
31. Matsuzawa Y, Ogawa H, Kimura K, et al. Renin-angiotensin system inhibitors and the severity of coronavirus disease 2019 in Kanagawa, Japan: a retrospective cohort study. *Hypertens Res*. 2020;43(11):1257-1266.
32. Gowrisankar YV, Clark MA. Angiotensin II regulation of angiotensin-converting enzymes in spontaneously hypertensive rat primary astrocyte cultures. *J Neurochem*. 2016;138:74-85.
33. Gallagher PE, Chappell MC, Ferrario CM, Tallant EA. Distinct roles for ANG II and ANG-(1–7) in the regulation of angiotensin-converting enzyme 2 in rat astrocytes. *Am J Physiol Cell Physiol*. 2006;290:C420-426.
34. Koka V, Huang XR, Chung AC, Wang W, Truong LD, Lan HY. Angiotensin II up-regulates angiotensin I-converting enzyme (ACE), but down-regulates ACE2 via the AT1-ERK/p38 MAP kinase pathway. *Am J Pathol*. 2008;172:1174-1183.
35. Wysocki J, Ye M, Batlle D. Plasma and kidney angiotensin peptides: importance of the aminopeptidase A/angiotensin III axis. *Am J Hypertens*. 2015;28:1418-1426.
36. Huang H, Baussant T, Reade R, Michel JB, Corvol P. Measurement of angiotensin II concentration in rat plasma: pathophysiological applications. *Clin Exp Hypertens A*. 1989;11:1535-1548.
37. Schulz A, Jankowski J, Zidek W, Jankowski V. Absolute quantification of endogenous angiotensin II levels in human plasma using ESI-LC-MS/MS. *Clin Proteomics*. 2014;11:37.
38. Patel VB, Clarke N, Wang Z, et al. Angiotensin II induced proteolytic cleavage of myocardial ACE2 is mediated by TACE/ADAM-17: a positive feedback mechanism in the RAS. *J Mol Cell Cardiol*. 2014;66:167-176.
39. Sriramula S, Cardinale JP, Lazartigues E, Francis J. ACE2 overexpression in the paraventricular nucleus attenuates angiotensin II-induced hypertension. *Cardiovasc Res*. 2011;92:401-408.
40. Gonzalez AA, Gallardo M, Cespedes C, Vio CP. Potassium intake prevents the induction of the renin-angiotensin system and

increases medullary ACE2 and COX-2 in the kidneys of angiotensin II-dependent hypertensive rats. *Front Pharmacol*. 2019;10:1212.

41. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. 2020;181:271-280.e8.
42. Wu C, Ye D, Mullick AE, et al. Effects of renin-angiotensin inhibition on ACE2 (angiotensin-converting enzyme 2) and TMPRSS2 (transmembrane protease serine 2) expression: insights into COVID-19. *Hypertension*. 2020;76:e29-e30.
43. Yamamoto K, Ohishi M, Katsuya T, et al. Deletion of angiotensin-converting enzyme 2 accelerates pressure overload-induced cardiac dysfunction by increasing local angiotensin II. *Hypertension*. 2006;47:718-726.
44. Zhu Y, Cui H, Lv J, et al. AT1 and AT2 receptors modulate renal tubular cell necroptosis in angiotensin II-infused renal injury mice. *Sci Rep*. 2019;9:19450.
45. Oller J, Alfranca A, Mendez-Barbero N, et al. C/EBPβ and nuclear factor of activated T cells differentially regulate adamts-1 induction by stimuli associated with vascular remodeling. *Mol Cell Biol*. 2015;35:3409-3422.
46. Marvar PJ, Goodman J, Fuchs S, Choi DC, Banerjee S, Ressler KJ. Angiotensin type 1 receptor inhibition enhances the extinction of fear memory. *Biol Psychiatry*. 2014;75:864-872.
47. Epelman S, Tang WH, Chen SY, Van Lente F, Francis GS, Sen S. Detection of soluble angiotensin-converting enzyme 2 in heart failure: insights into the endogenous counter-regulatory pathway of the renin-angiotensin-aldosterone system. *J Am Coll Cardiol*. 2008;52:750-754.
48. Glowacka I, Bertram S, Muller MA, et al. Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *J Virol*. 2011;85:4122-4134.
49. Pedersen KB, Sriramula S, Chhabra KH, Xia H, Lazartigues E. Species-specific inhibitor sensitivity of angiotensin-converting enzyme 2 (ACE2) and its implication for ACE2 activity assays. *Am J Physiol Regul Integr Comp Physiol*. 2011;301:R1293-1299.
50. Wang Y, Liu S, Liu H, et al. SARS-CoV-2 infection of the liver directly contributes to hepatic impairment in patients with COVID-19. *J Hepatol*. 2020;73:807-816.
51. Wichmann D, Sperhake JP, Lutgehetmann M, et al. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. *Ann Intern Med*. 2020;173:268-277.
52. Farkash EA, Wilson AM, Jentzen JM. Ultrastructural evidence for direct renal infection with SARS-CoV-2. *J Am Soc Nephrol*. 2020;31:1683-1687.
53. Puelles VG, Lutgehetmann M, Lindenmeyer MT, et al. Multiorgan and renal tropism of SARS-CoV-2. *N Engl J Med*. 2020;383:590-592.
54. Shibata S, Arima H, Asayama K, et al. Hypertension and related diseases in the era of COVID-19: a report from the Japanese Society of Hypertension Task Force on COVID-19. *Hypertens Res*. 2020;43:1028-1046.
55. Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nat Rev Rheumatol*. 2016;12:49-62.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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