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Angiotensin I Infusion Reveals Differential Effects of Angiotensin-Converting Enzyme in Aortic Resident Cells on Aneurysm Formation

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Abstract

Background: Angiotensin (Ang)I is cleaved by angiotensin-converting enzyme (ACE) to generate AngII. The purpose of this study was to determine the roles of ACE in endothelial and smooth muscle cells in aortic aneurysms.

Methods and Results: AngI infusion led to thoracic and abdominal aortic aneurysms in low-density lipoprotein receptor-deficient mice, which were ablated by ACE inhibition. Endothelial or

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Conflict of Interest
None.

smooth muscle cell-specific ACE deletion resulted in reduction of AngI-induced thoracic, but not abdominal, aortic dilatation.

Conclusions: AngI infusion causes thoracic and abdominal aortic aneurysms in mice. ACE in aortic resident cells has differential effects on AngI-induced thoracic and abdominal aortic aneurysms.

Keywords

Angiotensin; Angiotensin-converting enzyme; Aortic aneurysm; Endothelial cells; Smooth muscle cells

The renin-angiotensin system (RAS) plays a critical role in the development of thoracic and abdominal aortic aneurysms (TAAs and AAAs, respectively). Angiotensin (Ang)II is the principal effector of this system and is generated from AngI by the activity of angiotensin-converting enzyme (ACE). Therefore, it would be expected that pharmacological ACE inhibition would attenuate aneurysm formation; however, the efficacy of ACE inhibitors remains controversial. Several animal studies have suggested potentially beneficial effects of ACE inhibitors against AAAs,[1,2] but their effects on TAA formation vary according to animal models.[3,4] Clinically, one non-randomized study reported that an ACE inhibitor suppressed TAA growth in patients with Marfan syndrome,[5] whereas clinical evidence of effects of ACE inhibitors on AAAs is inconsistent.[6,7] Therefore, further studies are needed to clarify the effects of ACE on aneurysm formation.

Chronic AngII infusion in mice is an established model to define the mechanisms of TAAs and AAAs.[8,9] However, AngII is downstream of ACE in the RAS. Therefore, an aneurysm model using exogenous AngII administration is not optimal for investigating the role of ACE in the pathophysiology of aortic aneurysms.

In this study, we demonstrated TAA and AAA formation in hypercholesterolemic mice by subcutaneous infusion of AngI, the precursor of AngII. This mouse model enabled us to address the role of conversion of AngI to AngII in the pathophysiology of aortic aneurysms. Subsequently, using this mouse model, we also examined the effect of ACE deletion in either endothelial or smooth muscle cells (SMCs) on TAA and AAA formation.

Methods

Detailed methods are available in Supplementary File. Data supporting the findings reported in this manuscript are available from the corresponding authors upon reasonable request.

Results

AngI-Induced TAA and AAA in Mice

We first investigated the role of AngI in TAA and AAA formation in low-density lipoprotein receptor-knockout ($Ldlr^{-/-}$) mice. Subcutaneous AngI infusion led to expansion of the thoracic aorta in the ascending, but not descending, region (Figure 1A,B). Abdominal aortic diameter was also increased by infusion of AngI compared with the infusion of saline (Figure 1C,D). AAA formation was detected primarily in the suprarenal abdominal aorta

(Figure 1C). The regional specificity of aneurysm formation being dominant in the ascending and suprarenal abdominal regions was consistent with AngII-induced TAA and AAA mouse models.[8,9] In addition, the severity of AngI-induced thoracic and abdominal aortic dilation was equivalent to that observed following AngII infusion (Figure 2A,B). Because inflammation plays an important role in the development of aortic aneurysms, immunostaining of CD45, a leukocyte marker, was performed on aortic cross-sections from the ascending, descending thoracic, suprarenal, and infrarenal regions. AngI induced leukocyte accumulation predominantly in the adventitia (Supplementary Figure 1). AngI-induced leukocyte accumulation was detected in both disease-prone regions (ascending and suprarenal abdominal regions) and disease-resistant regions (descending thoracic and infrarenal aortas). Because ACE cleaves AngI to produce AngII, we then investigated the role of ACE in the development of AngI-induced TAAs and AAAs using enalapril, an ACE inhibitor. ACE inhibition significantly attenuated aneurysm formation in both the thoracic and abdominal regions (Figure 1A-D). These results support the notion that AngI induces TAA and AAA formation through an ACE-dependent mechanism to a severity that is comparable to that seen in the AngII-induced model.

Effects of ACE Deletion in Aortic Resident Cells on AngI-Induced TAAs and AAAs

ACE is related to AngI-induced aortic aneurysms and is abundant in endothelial cells. Therefore, we investigated endothelial cell-specific effects of ACE on aneurysm formation. AngI was infused in $Ldlr^{-/-}$ mice with *ACE* deletion in endothelial cells and in wild-type littermates. Endothelial-specific deletion of *ACE* led to mild reduction in AngI-induced ascending aortic dilation, whereas abdominal aortic dilation was not suppressed (Figure 3A,B). Because ACE is also present in SMCs, we next examined the effects of ACE in SMCs. Similar to *ACE* deletion in endothelial cells, SMC-specific deletion of *ACE* attenuated, in part, AngI-induced ascending aortic dilation, but had no effect on abdominal aortic dilation (Figure 3C,D). Thus, ACE in endothelial cells and SMCs individually has modest effects on AngI-induced TAAs, but has no effect on AngI-induced AAAs.

Effects of AngI Infusion on Aortic ACE Localization

Because ACE plays a role in the pathogenesis of AngI-induced TAAs and AAAs, we investigated localization of aortic ACE in the early phase of AngI infusion (5 days). ACE localization was examined in disease-prone and -resistant regions. ACE was abundant in the aortic intima and adventitia, with modest abundance in the media (Figure 4; Supplementary Figure 2). These abundance patterns were not different between disease-prone and -resistant regions and were not changed by AngI infusion.

Discussion

This study demonstrated that chronic subcutaneous AngI infusion induced formation of TAAs and AAAs, which comparable to the effects of AngII on aortic dilatations. The dilatation in thoracic, but not abdominal, aorta was partially attenuated by *ACE* deletion in either endothelial cells or SMCs.

The RAS plays a critical role in cardiovascular diseases, included aortic aneurysms and atherosclerosis. AngI is the inactive decapeptide in the RAS, and is converted into the active octapeptide AngII. We have previously reported the effects of AngI in the pathophysiology of atherosclerosis.[10] Chronic AngI infusion augmented atherosclerotic plaque size in hypercholesterolemic mice, which was inhibited by an ACE inhibitor. The present study showed the same effect on aneurysm formation: AngI infusion led to aortic aneurysm formation, which was suppressed by ACE inhibition. Importantly, the severity of AngI-induced atherosclerosis and aortic aneurysms is comparable to that of AngII-induced aortic phenotypes. These data support the notion that infused AngI is converted into AngII, thereby leading to aortic pathologies.

Pharmacological ACE inhibition prevented AngI-induced TAAs and AAAs, which provides solid evidence that ACE is the major enzyme to cleave AngI into AngII in this mouse model. In previous studies, we demonstrated that enalapril, an ACE inhibitor, exhibited comparative effects to losartan, an AngII receptor blocker (ARB), on atherosclerosis in mice.[11] However, no studies have compared effects of ACE inhibitors and ARBs on TAAs or AAAs. It would be important to compare these two classes of drugs on TAAs and AAAs side by side. Because AngII is produced by ACE, the TAA and AAA mouse models created by administration of exogenous AngII cannot be used to address the effects of ACE vs. angiotensin AT_1 receptors on aneurysm formation. The AngI-induced aneurysm mouse model is an optimal model to compare the effects of ACE inhibitors and ARBs.

In the present study AngI induced accumulation of leukocytes in the entire aorta, but AngI-induced aortic dilatation was predominantly located in the ascending and suprarenal abdominal regions. In addition, deletion of *ACE* in either endothelial cells or SMCs had modest effects on TAAs, but not AAAs. Therefore, AngI-induced aneurysm formation and the effects of vascular ACE are regionally specific. Although ACE plays a pivotal role in the pathogenesis of TAAs and AAAs, aortic ACE localization did not differ among regions and was not changed by AngI. Therefore, there is no direct evidence that ACE contributed to the regional specificity of AngI-induced TAAs and AAAs. A potential mechanism for this regional specificity is difference in the embryonic origins of SMCs. SMCs in the aorta have various origins, and these origins show unique distributions.[12,13] The regional specificity of TAAs corresponds to the distribution of SMC embryonic origins in the ascending aorta. Because different aortic regions exhibit different biological behaviors,[12] the distinct SMC origins provide a basis for this specific pathological feature. Another potential mechanism is the interaction between AngII and the AT_{1A} receptor. Whole-body AT_{1A} receptor deletion ablates AngII-induced TAA and AAA formation.[14,15] Surprisingly, deletion of the AT_{1A} receptor in SMCs had no significant effect on AngII-induced TAAs and AAAs, even though SMCs are a major cellular component of the aorta.[15,16] The AT_{1A} receptor is also expressed in aortic endothelial cells, but deletion of AT_{1A} receptors in endothelial cells had a modest effect on AngII-induced TAAs and no effect on AngII-induced AAAs.[15,16] Therefore, endothelial cells also have differential biological functions in different aortic regions, which may contribute to the regional specificity of TAA and AAA formation.

ACE is considered the principal enzyme responsible for converting AngI to AngII. In the present study, AngI-induced aortic aneurysms were ablated by ACE inhibition. Therefore,

AngI-induced aneurysms are formed through an ACE-dependent mechanism. However, deletion of *ACE* in either endothelial cells or SMCs had mild effects on TAAs, and no effect on AAAs, although endothelial cells and SMCs contribute to aortic ACE activity.[17] Thus, ACE in these cells has the potential to interact cooperatively to promote TAA and AAA formation. In addition to its presence in the aortic wall, ACE is abundant in many cell types and organs. Therefore, AngI could be converted to AngII by ACE in many cells and tissues, including subcutaneous and perivascular tissues. This may explain why ACE deletion in either endothelial cells or SMCs had no effect on AAA formation. Further studies are needed to clarify the kinetics of AngI conversion to AngII.

Several enzymes can convert AngI into AngII, including chymase and cathepsin G. Many studies have reported contributions of these enzymes to aneurysm formation. The abundance of chymase is increased in human TAA tissues,[18] and human AAA tissues exhibit increased expression of cathepsin G.[19] Genetic or pharmacological inhibition of these enzymes attenuates aneurysm formation in mice.[19,20] Because the efficacy of ACE inhibition is not consistent in the aortic aneurysm field,[1-7] these enzymes should be taken into account when studying mechanisms of TAAs and AAAs formation.

In summary, this study demonstrated equivalent effects of AngI and AngII on TAA and AAA formation that were mediated by the function of ACE. AngI-induced TAAs, but not AAAs, were partially attenuated by *ACE* deletion in aortic resident cells. The AngI-induced aortic aneurysm mouse model is optimal to explore the precise effects of ACE in the pathophysiology of TAAs and AAAs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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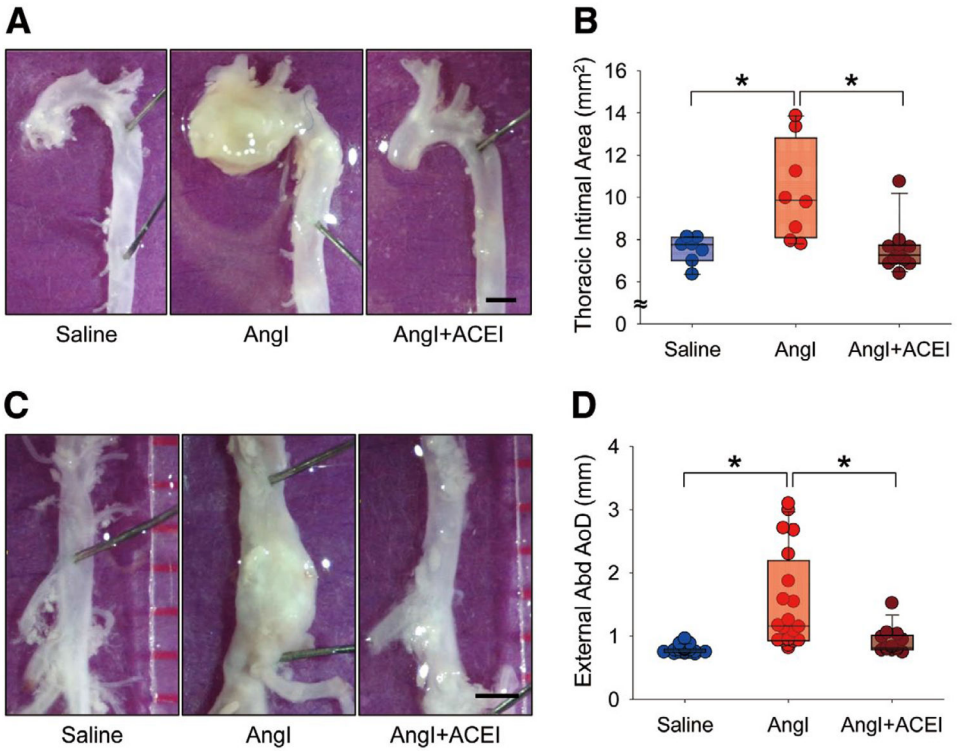

Figure 1.

Angiotensin (Ang)I-induced ascending and abdominal aortic dilatations that were ablated by angiotensin-converting enzyme (ACE) inhibition. (**A,C**) Representative gross appearance of thoracic (**A**) and abdominal (**C**) aortas. (**B**) Dilation of ascending aortas, determined by measuring the intima area. (**D**) Abdominal aortic dilation (Abd AoD), determined by measuring the maximal external diameters of the suprarenal aorta ex vivo. Boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the range (n=7–11 per group). *P<0.05 (Kruskal-Wallis test followed by Dunn's post hoc analysis). ACEI, ACE inhibitor (enalapril).

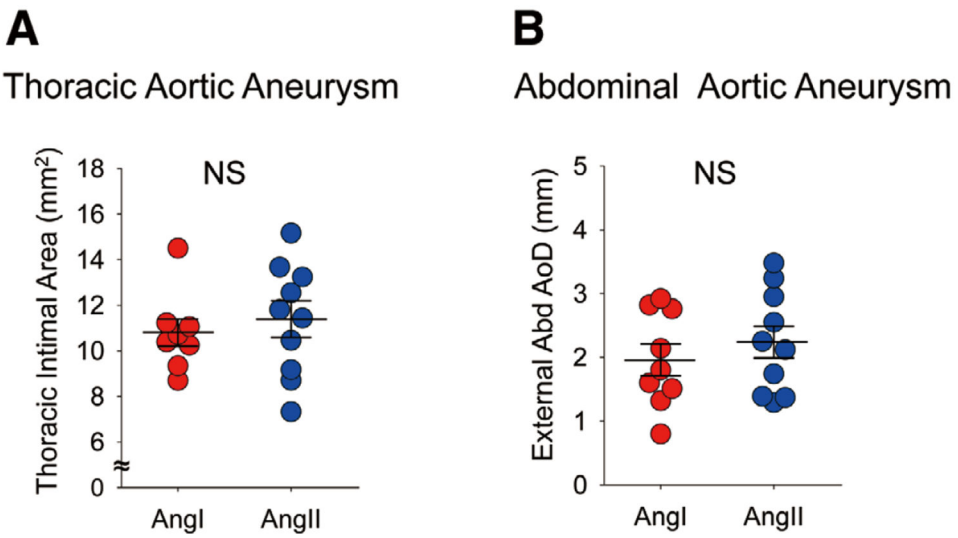

Figure 2.

Equivalent effects of angiotensin (Ang)I and AngII on the development of aortic aneurysms. (**A**) Intima area of the thoracic aorta and (**B**) external diameter of the abdominal aorta (Abd AoD) in AngI- and AngII-infused mice. Symbols show individual data points. The horizontal lines indicate mean values and whiskers indicate standard error of the mean (n=9–10 per group). There were no significant differences between groups (P>0.05, Student's t-test).

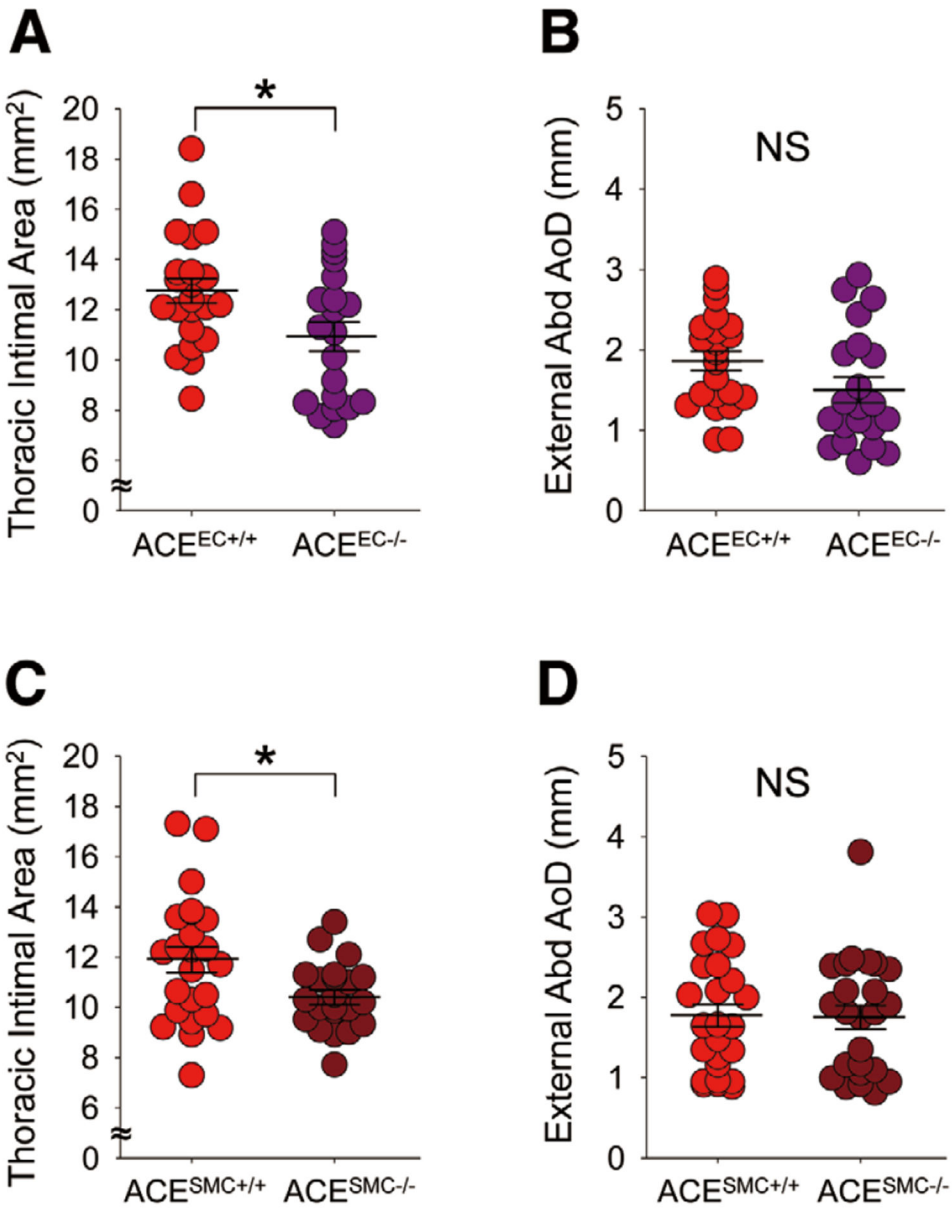

Figure 3.

Differential effects of angiotensin-converting enzyme (ACE) in aortic resident cells on angiotensin (Ang)I-induced formation of thoracic and abdominal aortic aneurysms (TAAs and AAAs, respectively). (**A**) Intima area of the thoracic aorta and (**B**) external diameter of the abdominal aorta (Abd AoD) in mice with deletion of *ACE* in endothelial cells. (**C**) Thoracic intima area and (**D**) maximal abdominal diameter (Abd AoD) in smooth muscle cell-specific *ACE* deficient mice. Symbols show individual data points. The horizontal lines indicate mean values and whiskers indicate standard error of the mean (n=24–25 per group). *P<0.05 (Welch's or Student's t-test).

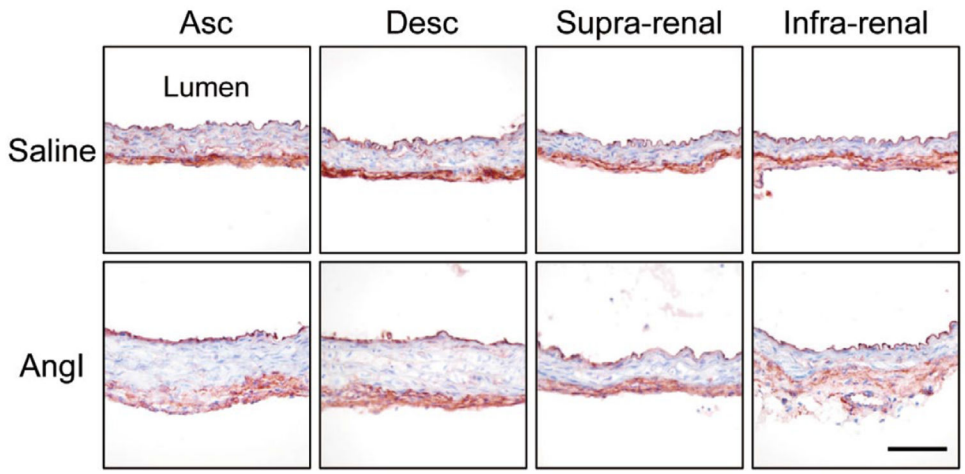

Figure 4.

Aortic angiotensin-converting enzyme (ACE) localization was not altered by angiotensin (Ang)I infusion. Representative images of ACE immunostaining in mouse aortas retrieved after 5 days of saline or AngI infusion. Aortic tissues were harvested from 4 regions: ascending (Asc) and descending (Desc) thoracic aortas and supra- and infrarenal aortas (n=3/group). Scale bar, 100 μm.