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

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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 smooth muscle cell-specific ACE deletion resulted in reduction of AngII-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 AngII-induced thoracic and abdominal aortic aneurysms.

Key Words: 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
AngII-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 AngII 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 AngII 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. 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 aorta) was consistent with AngII-induced TAA and AAA mouse models. 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 aorta).
Angiotensin I and Aortic Aneurysms

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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

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

![Figure 3](https://example.com/figure3.png)

**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).
inactive decapeptide in the RAS, and is converted into the active octapeptide AngII. We have previously reported the effects of Angl in the pathophysiology of atherosclerosis. Chronic Angl 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: Angl infusion led to aortic aneurysm formation, which was suppressed by ACE inhibition. Importantly, the severity of Angl-induced atherosclerosis and aortic aneurysms is comparable to that of Angll-induced aortic phenotypes. These data support the notion that infused Angl is converted into Angll, thereby leading to aortic pathologies.

Pharmacological ACE inhibition prevented Angl-induced TAAs and AAAs, which provides solid evidence that ACE is the major enzyme to cleave Angl into Angll in this mouse model. In previous studies, we demonstrated that enalapril, an ACE inhibitor, exhibited comparative effects to losartan, an Angll receptor blocker (ARB), on atherosclerosis in mice. 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 Angll is produced by ACE, the TAA and AAA mouse models created by administration of exogenous Angll cannot be used to address the effects of ACE vs. angiotensin AT1 receptors on aneurysm formation. The Angll-induced aneurysm mouse model is an optimal model to compare the effects of ACE inhibitors and ARBs.

In the present study Angl induced accumulation of leukocytes in the entire aorta, but Angll-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, Angl-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 Angl. Therefore, there is no direct evidence that ACE contributed to the regional specificity of Angl-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. 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, the distinct SMC origins provide a basis for this specific pathological feature. Another potential mechanism is the interaction between Angll and the AT1A receptor. Whole-body AT1A receptor deletion ablates Angll-induced TAA and AAA formation. Surprisingly, deletion of the AT1A receptor in SMCs had no significant effect on Angll-induced TAAs and AAAs, even though SMCs are a major cellular component of the aorta. The AT1A receptor is also expressed in aortic endothelial cells, but deletion of AT1A receptors in endothelial cells had a modest effect on Angll-induced TAAs and no effect on Angll-induced AAAs. 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 Angl to Angll. In the present study, Angl-induced aortic aneurysms were ablated by ACE inhibition. Therefore, Angl-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. 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, Angl could be converted to Angll 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 Angl conversion to Angll.

Several enzymes can convert Angll into Angl, 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, and human AAA tissues exhibit increased expression of cathepsin G. Genetic or pharmacological inhibition of these enzymes attenuates aneurysm formation in mice. Because the efficacy of ACE inhibition is not consistent in the aortic aneurysm field, these enzymes should be taken into account when studying mechanisms of TAAs and AAAs formation.

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

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Conflict of Interest
None.

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**Supplementary Files**

Please find supplementary file(s):
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