Smooth Muscle Cell Deletion of Low-Density Lipoprotein Receptor–Related Protein 1 Augments Angiotensin II–Induced Superior Mesenteric Arterial and Ascending Aortic Aneurysms

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Objective—Low-density lipoprotein receptor–related protein 1 (LRP1), a multifunctional protein involved in endocytosis and cell signaling pathways, leads to several vascular pathologies when deleted in vascular smooth muscle cells (SMCs). The purpose of this study was to determine whether LRP1 deletion in SMCs influenced angiotensin II–induced arterial pathologies.

Approach and Results—LRP1 protein abundance was equivalent in selected arterial regions, but SMC-specific LRP1 deletion had no effect on abdominal and ascending aortic diameters in young mice. To determine the effects of LRP1 deficiency on angiotensin II vascular responses, SMC-specific LRP1 (smLRP1+/+) and smLRP1-deficient (smLRP1−/−) mice were infused with saline, angiotensin II, or norepinephrine. Several smLRP−/− mice died of superior mesenteric arterial (SMA) rupture during angiotensin II infusion. In surviving mice, angiotensin II profoundly augmented SMA dilation in smLRP1−/− mice. SMA dilation was blood pressure dependent as demonstrated by a similar response during norepinephrine infusion. SMA dilation was also associated with profound macrophage accumulation, but minimal elastin fragmentation. Angiotensin II infusion led to no significant differences in abdominal aorta diameters between smLRP1+/+ and smLRP1−/− mice. In contrast, ascending aortic dilation was exacerbated markedly in angiotensin II–infused smLRP1−/− mice, but norepinephrine had no significant effect on either aortic region. Ascending aortas of smLRP1−/− mice infused with angiotensin II had minimal macrophage accumulation but significantly increased elastin fragmentation and mRNA abundance of several LRP1 ligands including MMP-2 and uPA.

Conclusions—smLRP1 deficiency had no effect on angiotensin II–induced abdominal aortic aneurysm formation. Conversely, angiotensin II infusion in smLRP1−/− mice exacerbatad SMA and ascending aorta dilation. Dilation in these 2 regions had differential association with blood pressure and divergent pathological characteristics. (Arterioscler Thromb Vasc Biol. 2015;35:00-00.)

Key Words: angiotensin II; aortic aneurysm; LRP1 protein, mouse

Low-density lipoprotein receptor–related protein 1 (LRP1) is a multifunctional member of the low-density lipoprotein receptor gene family that interacts with numerous ligands.1 Ligand engagement can result in either removal of the ligand from the extracellular environment or stimulation of specific intracellular signaling pathways.2 Determination of the contribution of LRP1 to vascular diseases was hindered initially by the embryonic lethality of whole body deletion.3 Subsequent vascular studies have demonstrated a role for LRP1 using cell-specific deletion, with particular emphasis on macrophages and smooth muscle cells (SMC). LRP1 deficiency in either of these cell types augments atherosclerosis in hypercholesterolemic mice and neointimal formation.4–8 SMC-specific deletion of LRP1 (smLRP1−/−) in aged mice also leads to several vascular phenotypes, including extended aortic length, aberrant superior mesenteric artery structure, and ascending aorta dilation.4,9,10

Recent studies have suggested a role for LRP1 in human vascular pathologies, particularly aortic aneurysms. Genome-wide association studies have implicated the rs1466535 polymorphism of the LRP1 gene with abdominal aortic aneurysms (AAAs), although the 2 studies differ
in which allele confers risk.\textsuperscript{11,12} A role for LRP1 in human AAAs has also been inferred by reduced abundance of LRP1 protein in aneurysmal tissue.\textsuperscript{13} In addition to an implied role in AAAs, exome sequencing of LRP1 identified a missense mutation in patients with thoracic aortic aneurysms who are afflicted with Marfan syndrome.\textsuperscript{14} These recent studies provide the basis for a potential role of LRP1 within aortic aneurysm formation.

Numerous studies have demonstrated a role for angiotensin II in several vascular pathologies, particularly atherosclerosis and aortic aneurysms.\textsuperscript{15–17} Angiotensin II is one of the few mediators, that regulates abundance of LRP1 protein in SMCs.\textsuperscript{18} In addition, angiotensin II regulates expression of many LRP1 ligands, some of which have been implicated in aneurysm formation and compromised vascular integrity. These include plasminogen activator inhibitor 1,\textsuperscript{19} transforming growth factor-\(\beta\),\textsuperscript{20} selected MMPs,\textsuperscript{21} and connective tissue growth factor.\textsuperscript{22} Given the potential for angiotensin II to augment many LRP1 ligands that affect vascular integrity, we speculated that LRP1 deficiency would influence vascular pathologies formed during chronic infusion of angiotensin II.

The purpose of this study was to determine whether the absence of SMC LRP1 influenced angiotensin II–induced arterial pathologies. The primary expectation was that smLRP1 deficient would promote angiotensin II–induced AAAs. However, this was not observed. In contrast, angiotensin II infusion profoundly augmented aneurysms in both the superior mesenteric artery (SMA) and ascending aorta. Despite the commonality of arterial dilation, the 2 regions differed markedly in response to elevated blood pressure and tissue pathology.

### Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

### Results

#### LRP1 Abundance in Arterial Vasculature

We first determined the abundance of LRP1 protein in selected arterial regions. LRP1 protein abundance was found to be uniform across all regions of the aorta and SMA (Figure 1A). Regional abundance of LRP1 protein was also determined in LRP1\textsuperscript{-/-} and LRP1\textsuperscript{+/-} mice to confirm that SM22-driven Cre effectively mediated recombination of the homozygous LRP1\textsuperscript{lox/lox} gene to prominently deplete the LRP1 gene in vascular SMCs (Figure 1B), as described previously.\textsuperscript{9} Baseline aortic measurements were acquired before initiating infusions, while smLRP1\textsuperscript{+/-} and smLRP1\textsuperscript{-/-} mice were \(\approx 8\) weeks of age, and these were predicted to have no overt vascular pathology. In agreement with this prediction, smLRP1 genotype had no significant difference in ascending or abdominal aortic diameters (Figure 1C and 1D).
SMC Depletion of LRPI Exacerbated SMA Dilation in a Blood Pressure-Dependent Manner

After baseline measurements, smLRP1+/+ and smLRP1−/− mice were infused with either saline or angiotensin II (1000 ng/kg per day) for 28 days. Chronic angiotensin II infusion increased systolic blood pressure in all mice, with no significant difference between genotypes (Table I in the online-only Data Supplement). Several smLRP1−/− mice died during angiotensin II infusion. On necropsy, mesenteric hematomas were noted (Figure 2A and 2B). In surviving mice, in vivo MRI demonstrated that angiotensin II–infused smLRP1−/− mice had pronounced SMA dilation (Figure 2C; Figure 1A and 1B in the online-only Data Supplement). Ex vivo measurements after 28 days of infusion demonstrated that SMA diameters of saline-infused smLRP1+/+ and smLRP1−/− mice were not significantly different (Figure 3A and 3B). In contrast, SMA diameters were dilated markedly during infusion of angiotensin II (P<0.05; Figure 3A and 3B).

Because angiotensin II infusion increased systolic blood pressure, we determined its contribution to the pronounced SMA dilation. smLRP1+/+ and smLRP1−/− mice were infused with either saline or norepinephrine (5.6 mg/kg per day) for 28 days. As described previously,23,24 this infusion rate of norepinephrine produced systolic blood pressure increases that were equivalent to angiotensin II infusion (Table I in the online-only Data Supplement). As with angiotensin II infusion, so also norepinephrine infusion promoted pronounced SMA dilation (Figure 3A and 3B).

Dilated SMA tissue was characterized by increased accumulation of CD68+ cells in the adventitia and media of smLRP1−/− mice infused with either angiotensin II or norepinephrine (Figure 3C, 3E, 3G, 3I, 3K, and 3M). In contrast, no CD68+ cells were detected in the media SMAs of...
smLRP1−/− mice that had been infused with saline, angiotensin II, or norepinephrine, although there was a sparse presence of CD68+ cells in the adventitia (Figure 3C, 3G, and 3K). Movat staining demonstrated a relatively modest, but significant, increase in elastin fragmentation in SMAs of smLRP1−/− mice after infusion of both angiotensin II and norepinephrine (Figure 3D, 3F, 3H, 3J, 3L, and 3N and Figure II in the online-only Data Supplement). In addition, angiotensin II infusion promoted the development of a pronounced neointima (Figure 3J).

Region-Specific Effects of SMC Depletion of LRP1 on Aortic Aneurysms

Aortic images were acquired in mice the day before termination using MRI. These images demonstrated that aortas were tortuous in smLRP1−/− mice, infused with angiotensin II (Figure IC, ID, and IIIA in the online-only Data Supplement). Aortic elongation in angiotensin II–infused smLRP1−/− mice was confirmed by ex vivo measurements, even though mice had the same body length (Figure IIIB and IIIC in the online-only Data Supplement). The aortic elongation was caused by increased length of both the thoracic and abdominal aorta (Figure IIID and IIIE in the online-only Data Supplement).

Aortic aneurysmal pathology was examined after 28 days of infusion. Against expectations, smLRP1−/− had no effect on angiotensin II–induced AAA formation as measured in vivo by ultrasound and ex vivo by measurement of diameters of suprarenal aortas (Figure IV in the online-only Data Supplement).

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Dimensions of the ascending aorta were also measured in both smLRP1−/− and smLRP1+/+ mice. As described previously,17,25 angiotensin II infusion significantly increased ascending aortic diameter in smLRP1−/− mice, independent of blood pressure elevation. This dilation was significantly exacerbated in smLRP1−/− mice infused with angiotensin II (P<0.05; Figure 4A and 4B). Unlike SMA dilation,
development of dilation in the ascending aorta was independent of systolic blood pressure because norepinephrine infusion did not significantly enhance expansion of ascending aortas from smLRP1−/− mice (Figure 4B).

Concomitant with luminal expansion were pathological changes in the media of ascending aortas after 28 days of angiotensin II infusion. No significant increase in CD68+ cell accumulation was discernible in aortic media of any group (Figure 5A, 5D, 5G, and 5J). However, CD68+ cell accumulation was visible in adventitia of the ascending aorta in angiotensin II–infused smLRP1−/− mice (Figure 5J). Elastin fragmentation was increased greatly in angiotensin II–infused smLRP1+/+ and smLRP1−/− mice compared with saline-infused mice. Angiotensin II–infused smLRP1−/− mice had the most significant increase in elastin disruption compared with all other groups (Figure 5B, 5C, 5E, 5F, 5H, 5I, and 5K–5M).

Angiotensin II Upregulated Expression of LRP1 Ligands in Ascending Aortas of smLRP1−/− Mice

To identify a potential mechanism by which angiotensin II exacerbates smLRP1−/− ascending aortic dilation, we quantified abundance of known LRP1 ligands. In these experiments, mRNA was extracted from ascending aortas of saline- or angiotensin II–infused smLRP1+/+ and smLRP1−/− mice and analyzed for mRNA abundance of LRP1 ligands. Given that norepinephrine infusion in smLRP1−/− mice did not produce ascending aortic dilation, we did not analyze expression of LRP1 ligands in these mice (Table II in the online-only Data Supplement). mRNA of several extracellular proteases including MMP-2 and uPA was increased significantly in angiotensin II–infused smLRP1−/− mice compared with saline-infused mice. Angiotensin II–infused smLRP1−/− mice had the most significant increase in elastin disruption compared with all other groups (Figure 5B, 5C, 5E, 5F, 5H, 5I, and 5K–5M).

Discussion

Angiotensin II infusion into mice promotes formation of aneurysms in the abdominal and ascending aortic regions, and augments atherosclerosis when associated with hypercholesterolemia.15–17 Although these vascular pathologies are caused by the interaction of angiotensin II with AT1a receptors,26–28 yet, it has to be defined which cell type is stimulated by angiotensin II to form these diseases, except in the case of deleting endothelial AT1a receptors on the development of ascending aortic aneurysms.26,29,30 LRP1 is expressed in many cell types where it exerts protective effects from vascular pathology. Angiotensin II regulates many LRP1 ligands, but the interactions of angiotensin II, LRP1, and vascular disease have not been studied in vivo. The present study demonstrated that LRP1 protein abundance was equivalent throughout all arterial regions examined. Surprisingly, deletion of LRP1 in SMCs had no effect on AAA, whereas it promoted angiotensin II–induced SMA and ascending aortic aneurysms by disparate mechanisms.

Many studies have reported aortic aneurysm formation during the initial few days of angiotensin II infusion. Death is commonly attributable to rupture of the suprarenal or ascending aorta.11,12 Although arterial rupture also occurred in a limited number of smLRP1−/− mice during angiotensin II infusion, this was attributed to SMA rupture rather than compromise of aortic integrity. Although SMA enlargement has been described previously in aged smLRP1−/− mice,33 it has not been documented during angiotensin II infusion. To further define the differences between SMA and aortic aneurysms in smLRP1−/− mice, we investigated the effects of hypertension on aneurysmal disease within these arterial regions. It has been previously demonstrated that increases of blood pressure are not directly involved in angiotensin II–augmented aneurysmal formation of the suprarenal and ascending aortic

Figure 4. Smooth muscle cell–specific LRP1–deficient (smLRP1−/−) mice exacerbated angiotensin (Ang) II–induced ascending aortic aneurysm formation independent of systolic blood pressure. A, Representative intact aortic arches of smLRP1+/+ and smLRP1−/− infused with saline, Ang II, or norepinephrine (NE). B, Ascending aortic diameter growth in saline, Ang II, or NE-infused smLRP1+/+ and smLRP1−/− mice. Histograms represent group means (n=5 per group), and errors are SEM. *P<0.05 for Ang II–infused smLRP1+/+ mice compared with saline or NE-infused smLRP1+/+ or smLRP1−/− mice by 2-way ANOVA. Multiple comparisons were performed using Holm–Sidak test. #P<0.05 for Ang II–infused smLRP1−/− vs all other groups by 2-way ANOVA. ns indicates no statistical significance when comparing within a given infusion group.
regions. This conclusion was primarily based on comparisons with pathology in mice with hemodynamic equivalent infusions of norepinephrine.6,23,25 Using the same strategy in this study, hemodynamic equivalent infusions of either angiotensin II or norepinephrine promoted a similar extent of dilation of the SMA, whereas infusion of norepinephrine did not promote ascending or abdominal aortic dilation. Therefore, the role of blood pressure on SMA dilation differs from aneurysm formation in aorta.

Furthermore, the pathological characteristics differed between tissues in angiotensin II–induced SMA dilation and aorta aneurysm. SMAs retrieved from smLRP1−/− mice with increased systolic blood pressure had greatly expanded thickness, much of which was because of an area on the luminal aspect that was not encased by elastin layers and was assumed to be neointimal. A markedly thickened SMA has also been demonstrated in hypercholesterolemic smLRP1−/− mice through an undefined mechanism.3,33 Mechanistic studies have focused on LRP1 signaling through PDGFR-β, and its antagonism by a tyrosine kinase inhibitor, Gleevec, decreased atherosclerosis. However, the deletion of PDGFR-β had no effect on SMA dilation.33

SMAs from angiotensin II–infused smLRP1−/− mice also contained abundant macrophages in all regions of tissue sections. Elastin fragmentation products have been invoked as a potential mechanism of promoting macrophage infiltration.34 Therefore, the presence of elastin fragmentation in angiotensin II– and norepinephrine-infused mice could be responsible for the pronounced macrophage accumulation. However, this relationship differed in the ascending aorta of angiotensin II–infused mice where there was extensive elastin fragmentation but minimal vascular macrophage accumulation. The cause and consequence of macrophage accumulation in the SMA need further study.

Previous studies in mice have inferred that LRP1 deficiency led to formation of AAAs,3 but did not provide direct evidence. In humans, LRP1 has been linked to the development of AAAs on the basis of genetic associations and changes of LRP1 protein abundance in aneurysm tissue.11–13 Therefore, it was predicted that SMC deletion of LRP1 would promote development of AAAs in normocholesterolemic mice during angiotensin II infusion. Because normocholesterolemic mice usually have a low incidence of angiotensin II–induced AAAs,35–37 they provided a model to determine augmentation of AAAs. Contrary to this prediction, LRP1 absence did not lead to overt AAA formation during angiotensin II infusion, or any measurable change in aortic diameters compared with LRP1-proficient mice. LRP1 was effectively deleted in this aortic region, so the absence of alteration in AAA pathology was not attributable to any technical shortcomings. This lack of effect may not necessarily contradict the inference of LRP1 in human AAA, which is derived from weak associations that have generated discrepant allelic associations.

There has been considerable interest on the role of angiotensin II in the development of ascending aortic aneurysms, both experimentally and clinically.38 Interest was stimulated primarily by demonstrating that an AT1 receptor antagonist, losartan, prevented thoracic aortic aneurysms in mice harboring a C1039G fibrillin-1 mutation.39 Conversely, angiotensin II infusion promotes expansion of the ascending aorta in both normo- and hypercholesterolemic mice.17,25 SMC-specific LRP1 deficiency also promotes expansion of the aortic root and descending aorta in aged mice.40 The present study demonstrated that lack of SMC LRP1 greatly augmented angiotensin II–induced ascending aortic expansion. As described for angiotensin II–induced ascending aortic dilation in LRP1-proficient mice,40 norepinephrine infusion at rates that induced equivalent systolic blood pressure increases did not mimic effects of angiotensin II. Also, although LRP1 deficiency greatly augmented rates of ascending aortic expansion, there was no profound difference in pathological characteristics of tissue sections. These were characterized by profound elastin degradation, but a relative paucity of medial macrophage accumulation. Other characteristics include the lack of any neointimal formation and a predominance of medial changes.

Figure 5. Ascending aortic sections from angiotensin (Ang) II–infused smooth muscle cell–specific LRP1–deficient (smLRP1−/−) mice have increased elastin fragmentation. A to L, Representative ascending aortic sections of smLRP1−/− (A–C, G–I) or smLRP1−/− mice (D–F, J–L) infused with saline or Ang II infusion. Macrophage accumulation was identified by CD68+ cells (A, D, G, J). Additional higher magnification of Movat stained sections is seen in the green boxes (C, F, I, L). Sections are oriented with the lumen at the top of the image. Yellow arrows indicate regions of marked elastin fragmentation. M, Elastin fragmentation counted in cross-sections of ascending aortas from saline or Ang II–infused smLRP1−/− and smLRP1−/− mice. Histobars represent group means (n=4 per group), and errors are SEM. Lines represent P<0.05 by 2-way ANOVA followed by a post hoc Holm–Sidak test.
on the adventitial aspect of the aorta. Overall, although concomitant angiotensin II infusion and SMC-specific LRP1 deficiency promote dilation of the ascending aorta and SMA, the disparate roles of blood pressure and pathological appearances imply different mechanisms for aneurysm formation.

The mechanism by which LRP1 deficiency enhances selected angiotensin II–induced vascular pathologies is unclear. Angiotensin II generates vascular pathologies through stimulation of AT1a receptors, but deletion of this receptor in SMCs had no effect on angiotensin II–induced atherosclerosis stimulation of AT1a receptors, but deletion of this receptor in SMCs had no effect on angiotensin II–induced atherosclerosis. Therefore, it is unclear whether the increased abundance of specific mRNAs is a cause or consequence of aneurysm formation. Also, previous studies using smLRP1−/− mice noted increased mRNA abundance, implying that other proteases from mice that had been chronically infused with angiotensin II to examine specific LRP1 ligands that are enhanced by angiotensin II to compromise vascular integrity.

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**Disclosures**

None.

**References**

9. Muratoglu SC, Belgrave S, Hampton B, Migliorini M, Coksaygan T, Chen L, Mikhailenko I, Strickland DK. LRP1 protects the vasculature by...


**Significance**

Low-density lipoprotein receptor–related protein 1 (LRP1) is a multifunctional protein that engages many ligands to perform endocytosis and signaling. It has been implicated in vascular pathologies in both humans and mice. Angiogenins II is well known to promote experimental vascular pathologies. In this study, the effects of LRP1 deletion in smooth muscle cells were determined during angiogenin II–induced vascular pathologies. Unexpectedly, angiogenin II infusion led to deaths because of superior mesenteric arterial rupture that was associated with pronounced dilatation of this vessel in smooth muscle cell–specific deficient LRP1−/− mice. Also unexpectedly, there was no effect of smooth muscle cell deficiency of LRP1 on formation of abdominal aortic aneurysms during angiogenin II infusion. However, the absence of LRP1 led to a major promotion of ascending aortic dilatation during angiogenin II infusion. Therefore, angiogenin II infusion in mice deficient of LRP1 in smooth muscle cells had a highly regional effect on development of vascular pathologies.