Effects of Endogenous Angiotensin II on Abdominal Aortic Aneurysms and Atherosclerosis in Angiotensin II-infused Mice

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Abbreviations:

AAA:	Abdominal aortic aneurysm
ACE:	Angiotensin-converting enzyme
Ang:	Angiotensin
LS-MS/MS:	Liquid chromatography-mass spectrometry/mass spectroscopy

Abstract:
Angiotensin II (AngII), a major effector of the renin-angiotensin system, exerts critical roles in regulating vascular function. AngII infusion induces abdominal aortic aneurysms (AAAs) and exacerbates atherosclerosis in hypercholesterolemic mice. We determined the effects of AngII infusion on endogenous AngII regulation and AngII-mediated AAAs and atherosclerosis. AngII infusion increased renal, but not plasma, AngII concentrations in male mice. AngI concentrations were decreased modestly in kidney, but more profoundly in plasma, during AngII infusion. Bovine AngII (DRVYVHPF) has one amino acid difference from mouse AngII (DRVYIHPF) that can be distinguished by LC-MS/MS to determine exogenous versus endogenous peptides. AngII infusion reduced endogenous renal AngII concentrations. To determine whether the residual endogenous AngII exerted an effect on exogenous AngII-mediated AAAs and atherosclerosis, aliskiren (a direct renin inhibitor) was administered to AngII-infused male LDL receptor deficient mice. Although aliskiren did not attenuate AAAs in AngII-infused mice, atherosclerotic lesion size was reduced. In conclusion, endogenous AngII concentrations are reduced during AngII infusion but still contribute to atherosclerosis, but not AAA, in AngII-infused hypercholesterolemic mice.

Angiotensin (Ang) II is a major effector of the renin-angiotensin system and important in regulating vascular function. Infusion of AngII induces abdominal aortic aneurysms (AAAs) and exacerbates atherosclerosis in hypercholesterolemic mice. In AngII-infused normocholesterolemic rats, endogenous AngII production remains in kidney.[1] Effects of endogenous AngII on AAAs and atherosclerosis during AngII infusion in hypercholesterolemic mice have not been studied.

Either vehicle or murine AngII (1,000 ng/kg/min) was infused into male C57BL/6J mice for 7 days. Our previous studies revealed that liver-specific deletion of angiotensinogen, the sole precursor of AngII, reduced atherosclerotic lesion area with decreases of AngII concentrations in kidney, but not in plasma.[2] In addition, inhibition of angiotensinogen uptake into renal cells ameliorated atherosclerosis development.[3] These results indicate an important role of renal AngII in atherosclerosis formation. Thus, we determined concentrations of angiotensin peptides in plasma and kidney by liquid chromatography-mass spectrometry/mass spectroscopy (LS-MS/MS). Plasma AngII concentrations were not altered by AngII infusion (**Figure A**). Consistent with a previous report,[1] renal AngII concentrations were significantly higher in AngII-infused mice than in vehicle-infused mice (**Figure A**). Since AngII is metabolized to AngIII and Ang(1-7) and these peptides contribute to the pathophysiology of atherosclerosis and hypertension,[4] we assessed AngIII and Ang(1-7) concentrations in AngII-infused mice. Ang(1-7) was not detectable in plasma and kidney from either vehicle- or AngII-infused mice, and AngIII concentrations were not different in plasma and kidney between infusions (**Figure B**). These results indicate that exogenous AngII does not affect productions of major AngII metabolites.

AngI is the direct substrate of AngII, which is cleaved by angiotensin-converting enzyme (ACE). Therefore, we measured plasma and renal AngI concentrations. AngII infusion decreased AngI concentrations in both plasma and kidney (**Figure C**). However, AngII-induced reduction of AngI concentrations was modest in kidney compared to those in plasma (96% in plasma vs 62 % in kidney, $p<0.05$ by Mann-Whitney U test, **Figure C**), indicating persistent renal AngII production during AngII infusion. We next investigated the presence of endogenous AngII in kidney of AngII-infused mice. Bovine AngII differs from murine AngII (Asp-Arg-Val-Tyr-**Ile**-His-Pro-Phe) in the fifth amino acid being Val (Asp-Arg-Val-Tyr-**Val**-His-Pro-Phe). The one amino acid difference results in a mass difference that enables distinction between bovine and murine AngII by LC-MS/MS as exogenous and endogenous sources, respectively, in bovine AngII-infused mice (**Figure D**). We verified comparable effects of bovine AngII with mouse AngII on AAA and atherosclerosis formation in hypercholesterolemic mice (data not shown). In vehicle-infused mice, the median of renal endogenous AngII concentrations was 843 pg/g (interquartile range: 669-1179 pg/g). AngII infusion reduced endogenous AngII concentrations to 161 pg/g (interquartile range: 77-235 pg/g). Endogenous AngII was still detectable in mice with bovine AngII infusion (**Figure D**). Alongside the presence of renal AngI, these data support the notion that intrarenal AngII production is continued during AngII infusion.

We next investigated whether inhibition of endogenous AngII production attenuates AAA and atherosclerosis formation during AngII infusion. ACE inhibitors or direct renin inhibitors suppress endogenous AngII production. However, ACE inhibitors target several other substrates such as bradykinin that may affect cardiovascular functions. To investigate effects of endogenous AngII on AAA and atherosclerosis formation, the present study used aliskiren, a direct renin inhibitor, to inhibit endogenous AngII production. Based on our previous study that aliskiren infusion of 25 mg/kg/day led to maximal inhibitory effects on endogenous AngII production in mice,[5] we used this infusion rate of aliskiren in AngII-infused male LDL receptor deficient mice. As expected, AngII infusion induced AAAs and augmented atherosclerotic lesions compared to vehicle infusion (**Figure E, F**). Aliskiren did not inhibit abdominal aortic expansion in AngII-infused mice (**Figure E**). Although atherosclerotic lesion size in aliskiren and AngII-infused mice was larger than vehicle-infused mice, aliskiren reduced lesion size significantly, compared to AngII infusion alone (**Figure F**). These results support the concept that AAA development was attributed mainly to exogenous AngII, whereas atherosclerosis was augmented by both endogenous and exogenous AngII.

In conclusion, renal endogenous AngII is present in AngII-infused mice, and endogenous AngII contributes to AngII-mediated atherosclerosis, but not AAA, formation in hypercholesteremic mice.

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Disclosures
None

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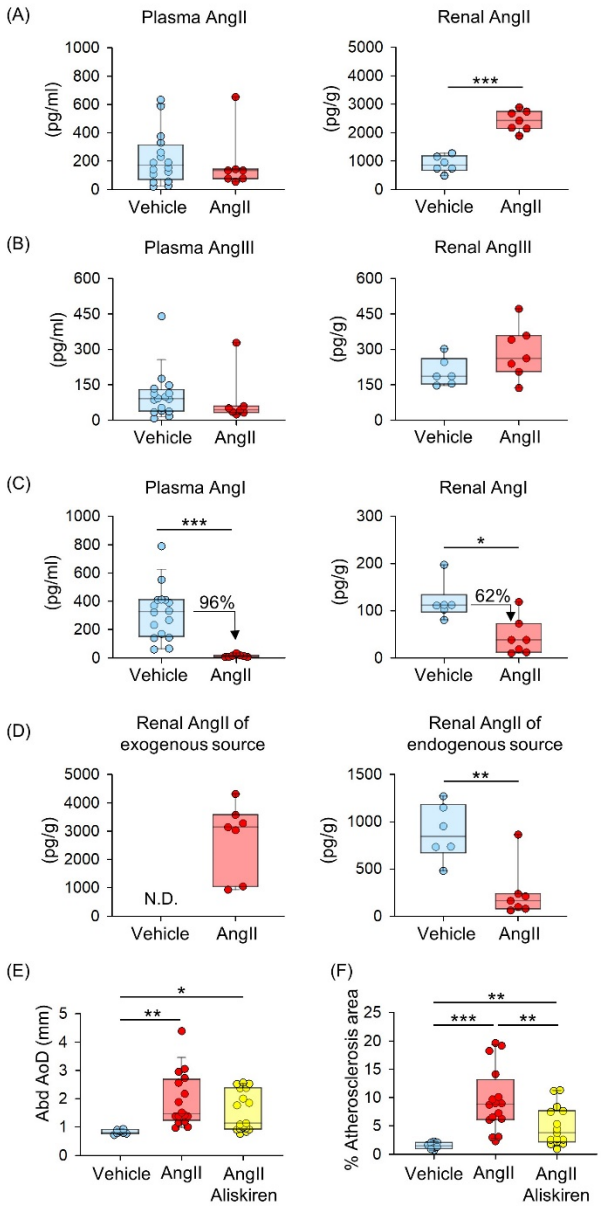

Figure. Contributions of endogenous AngII to AAAs and atherosclerosis in AngII-infused mice. Plasma and renal concentrations of **(A)** Ang II, **(B)** AngIII, and **(C)** AngI in male C57BL/6J mice (10-week-old, The Jackson Laboratory, stock #000664) infused with either vehicle or murine AngII (1,000 ng/kg/min) for 7 days. n=7-16/group. **(D)** Renal AngII concentrations of exogenous and endogenous source in either vehicle or bovine AngII (1,000 ng/kg/min) infused mice. n=7-15/group. **(E)** Maximal external diameters of abdominal aorta and **(F)** percent atherosclerotic lesion areas in male LDL receptor deficient mice (8-week-old; The Jackson Laboratory, stock #002207) fed Western diet and infused with vehicle, murine AngII (1,000 ng/kg/min), or both murine AngII and aliskiren (25 mg/kg/day). n=8-17/group. Angiotensin peptides were measured by LC-MS/MS. Data are presented as box plots drawn from the 25th to 75th percentiles with a line at the median. *p<0.05, **p<0.01, ***p<0.001 by Mann-Whitney U test or Kruskal-Wallis with Dunn's method. N.D. indicates not detectable.