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ORIGINAL ARTICLE

Azilsartan Is Associated With Increased Circulating Angiotensin-(1–7) Levels and Reduced Renovascular 20-HETE Levels

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BACKGROUND
Activation of angiotensin (ANG) II type 1 receptors (AT_1R) promotes vasoconstriction, inflammation, and renal dysfunction. In this study, we addressed the ability of azilsartan (AZL), a new AT_1R antagonist, to modulate levels of plasma ANG-(1–7) and renal epoxyeicosatrienoic acids (EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE).

METHODS
Sprague-Dawley rats were infused with ANG II (125 ng/min) or vehicle (VEH). AZL (3 mg/kg/day) or VEH was administered starting 1 day prior to ANG II or VEH infusion. On day 10, plasma was obtained for measurement of ANG-(1–7) and kidneys for isolation of microvessels for EET and 20-HETE determination and histological evaluation.

RESULTS
Mean 24-hour blood pressure (BP) was not different between VEH and AZL treatment groups, whereas the BP elevation with ANG II infusion (121 ± 5 mm Hg) was completely normalized with AZL cotreatment (86 ± 3 mm Hg). The ANG II-induced renal damage was attenuated and cardiac hypertrophy prevented with AZL cotreatment. Plasma ANG-(1–7) levels (pg/ml) were increased with AZL treatment (219 ± 22) and AZL + ANG II infusion (264 ± 93) compared to VEH controls (74.62 ± 8). AZL treatment increased the ratio of EETs to their dihydroxyeicosatrienoic acid (DHET) metabolites and reduced 20-HETE levels.

CONCLUSIONS
Treatment with AZL completely antagonized the elevation of BP induced by ANG II, prevented cardiac hypertrophy, attenuated renal damage, and increased ANG-(1–7) and EET/DHET ratio while diminishing 20-HETE levels. Increased ANG-(1–7) and EETs levels may emerge as novel therapeutic mechanisms contributing to the antihypertensive and antihypertrophic actions of AZL treatment and their relative role compared to AT_1R blockade may depend on the etiology of the hypertension.

Keywords: angiotensins; AT1 receptor blocker; blood pressure; eicosanoids; hypertension; kidney.

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The pathophysiology of hypertension involves a complex interaction of multiple vascular effectors including the activation of the sympathetic nervous system, the renin–angiotensin–system (RAS) and inflammatory mediators.[1,2] Subsequent vasoconstriction and inflammation ensue, leading to vessel wall remodeling and, finally, to the formation of atherosclerotic lesions as the hallmark of advanced disease. The kidney is an important target for the actions of the RAS and contains a complete local RAS that expresses the bioactive peptides angiotensin II (ANG II) and ANG-(1–7). ANG II, angiotensin I-converting enzyme (ACE), and ANG II subtype 1 (AT_1) receptor activation initiate pathophysiological consequences by promoting renal dysfunction, morphologic injury, and inflammation.[3,4] The discovery that AT_2 receptors and ANG-(1–7) oppose the pressor, oxidative, proliferative, profibrotic, and prothrombotic actions mediated by ANG II has contributed to the understanding that the RAS is composed of opposing arms: the pressor arm constituted by the enzyme ACE, ANG II as the product, and the AT_1 receptor as the main protein mediating the biological actions of ANG II; the second arm is composed of AT_2 receptors that oppose, in part, the actions mediated by AT_1 receptors, and the third arm mediated by ANG-(1–7), produced through hydrolysis of ANG I and ANG II, and the *Mas* receptor as the protein conveying the vasodilator, antiproliferative, antifibrotic, and antithrombotic effects.[5–7] Both AT_2 receptor activation and ANG-(1–7) play a beneficial role in renal disease models. In an experimental model of renal hypertension, the administration of a selective ANG-(1–7) receptor blocker or an inhibitor of ACE2, the enzyme responsible for the conversion of ANG II to ANG-(1–7), was associated with worsening of hypertension and renal function, whereas treatment with ANG-(1–7) prevented development of severe hypertension and end-organ damage in spontaneously hypertensive rats.[8–10]

Unlike ACE inhibitors, an AT_1 receptor antagonist results in increased ANG II levels and activation of AT_2 receptors.[11,12] ANG II is also an excellent substrate for ACE2 and

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is efficiently metabolized to ANG-(1–7), which stimulates *Mas* receptors. By interacting with the *Mas* receptor, ANG-(1–7) induces the release of nitric oxide (NO) from endothelial cells and thereby counteracts the effects of ANG II.[13,14] Taken together, the ACE2–ANG-(1–7)–*Mas* axis emerges as a novel therapeutic target for decreasing cardiovascular diseases.

The cytochrome P450 (CYP)-derived arachidonic acid (AA) metabolites, the 4 regioisomeric *cis*-epoxyeicosatrienoic acids (EETs)—5,6-, 8,9-, 11,12-, and 14,15-EETs—and 20-hydroxyeicosatetraenoic acid (20-HETE), generated by epoxygenases and hydroxylases, respectively, occupy a key position in the regulation of renal vascular tone.[15,16] EETs are important modulators of cardiovascular function and have been recognized for their vasodilator, natriuretic, anti-inflammatory, antiproliferative, and profibrinolytic properties. The contribution of EETs to blood pressure (BP) regulation has been established in several different animal models and an increase in the production of EETs is one of the significant components of the kidney's adaptive response to prevent elevation of BP.[17] Recently, AT_2 receptor-coupled signaling has been shown to increase EET synthesis. In contrast, although 20-HETE also promotes natriuresis, it exhibits prohypertensive actions, by increasing myogenic tone and promoting endothelial dysfunction and vascular smooth muscle proliferation.[18] In addition, 20-HETE induces endothelial ACE, and ANG II infusion increases microvascular 20-HETE levels.[19–21]

We have reported that ANG II-induced hypertension alters renal microvascular CYP-AA metabolites formation.[20] In this study, we addressed the role of CYP-AA metabolites in contributing to the activity of a newly developed AT_1R blocker (ARB), azilsartan (AZL), to reverse ANG II-induced hypertension and renal injury. In addition, as not all ARBs have the same biological activities, we addressed the influence of AZL on other vasodilator pathways including circulating levels of ANG-(1–7) and urinary NO excretion both in the presence and absence of infused ANG II.[22]

METHODS

Animals

Male Sprague-Dawley rats weighing 175–200 g (6–7 week old; Charles River) were fed Purina Laboratory diet 5001 and used in accordance with National Institutes of Health guidelines. The New York Medical College Institutional Animal Care and Use Committee approved all experimental protocols.

Measurement of BP

BP (systolic (SBP), diastolic (DBP), and mean (MBP)) and heart rate were continuously monitored by radiotelemetry. Transoma (Data Sciences International) radiotelemetry probes for small animals (TA11PAC40) were implanted into the rats at 6–7 weeks of age using sterile surgical techniques, as described.[17,23] Animals were allowed 7 days to recover from the surgery and acclimate to the experimental environment. BP and heart rate were sampled over a 10-second period at 10-minute intervals for the duration of the study. Transmitted data were collected using a computer-driven data acquisition system (Dataquest IV, DSI).

In vivo administration of drugs

The renoprotective mechanism(s) of AZL were explored using an established ANG II infusion protocol, which results in reproducible increases in BP and renal damage.[20,24] One week after implantation with radiotelemetry probes, the rats were randomly divided among 4 treatment groups—group 1: vehicle by minipump and vehicle by gavage (n = 8; VEH), group 2: vehicle by minipump and AZL medoxomil by gavage (n = 8; AZL), group 3: ANG II infusion by minipump + vehicle by gavage (n = 8; ANG II), and group 4: ANG II infusion by minipump + AZL medoxomil by gavage (n = 8; ANG II + AZL). AZL medoxomil treatment was started 1 day prior to infusion of ANG II or vehicle, by daily gavage at a dose of 3 mg/kg and vehicle controls received 3 ml/kg of 0.5% carboxymethylcellulose. This dose of AZL medoxomil and dosing protocol have been reported to reduce myocardial infarction size.[25]

Osmotic minipumps implantation

Following 1 week of recovery, osmotic minipumps were implanted subcutaneously, under sterile conditions, into rats anesthetized with isoflurane. The rats in group 3 and 4 were infused with ANG II (125 ng/min/kg; Sigma–Aldrich) via an Alzet minipump and released at a rate of 0.5 µl/h. Animals in groups 1 and 2 were infused with the vehicle for ANG II (0.01 N acetic acid) at 0.5 µl/h. After 10 days of infusion, the animals were anesthetized for collection of plasma and excision of the heart and kidneys.

ANG-(1–7) and NO measurements

On day 10, rats were anesthetized with sodium pentobarbital (65 mg/kg, IP) and blood (~3 ml) was collected from the abdominal aorta into chilled tubes containing a mixture of peptidase inhibitors: 25 mmol/l ethylenediaminetetraacetic acid, 0.44 mmol/l 1,20-orthophenanthrolene monohydrate (Sigma Chemical, St. Louis, MO), 1 mmol/l sodium *para*-chloromercuri-benzoate, and 3 µmol/l WFML (rat renin inhibitor acetyl-His-Pro-Phe-Val-Statine-Leu-Phe).[26] Blood samples were centrifuged and plasma re-centrifuged at 2,000 rpm for 10 min at 4 °C. Plasma samples were stored at −80 °C for measurement of ANG-(1–7) levels by radioimmunoassay (The Hypertension Core Laboratory, Wake Forest University of Medicine).[12]

Urinary NO levels were measured as the sum of nitrite/nitrate levels and determined by the Greiss reaction using an ELISA kit (Cayman Chemicals, Ann Arbor, MI) following the manufacturer's instructions. Urine samples were diluted by 1/50, as urine contains relatively high levels of nitrate. The plate was read using a microplate reader at absorbance of 540 nm.

Lipid analysis

Preglomerular microvessels were microdissected and total lipids extracted and analyzed as described previously.[17] The extracts were subjected to alkaline hydrolysis to release esterified CYP-AA metabolites and CYP-AA metabolites quantified with a Q-trap 3200 linear ion trap quadrupole liquid chromatography/tandem mass spectrometry equipped with a Turbo V ion source operated in negative electrospray mode (Applied Biosystems). Synthetic standards were used to obtain standard curves (5–500 pg) for each eicosanoid (linear regression R^2 values: >0.99) and internal standards.

Histopathological examination

Coronal sections of kidney, cut at 3–4 mm, were preserved in 10% phosphate-buffered formalin, and at least 3 such tissues were sampled from different regions and embedded in blocks of paraffin. Histologic sections (2–3 μm) were stained with hematoxylin and eosin and examined by light microscopy at ×50 and ×100 in a blinded manner for lesions, as described previously.[27] Vascular damage was assessed for thrombotic and/or proliferative arteriopathy. Proliferative arteriopathy was characterized by nodular mural thickening as a result of proliferation of markedly swollen myointimal cells often superimposed on thrombotic lesions. Tubules were assessed for casts and ischemic profiles. Ischemic tubules were characterized by relatively small diameter, intact but wrinkled basement membranes, and lined by simplified epithelium.

Statistical analysis

All data are expressed as means ± SEM. One-way analysis of variance followed by the Bonferroni *post hoc* test was used when multiple comparisons were made. Blood pressure measurements were analyzed using repeated measures analysis of variance followed a Bonferroni *post hoc* test. Unpaired *t*-test analysis was used for all other data. A P value of <0.05 was considered statistically significant.

RESULTS

Blood pressure response to ANG II infusion and azilsartan treatment

Systolic, diastolic, and mean BP were significantly increased by day 3 of ANG II infusion and steadily continued until a maximum BP was seen at days 7–8 (Figure 1). Consistent with the higher BP, urine volume was significantly increased in the ANG II-infused group (Figure 2). The BP elevation and diuresis, induced by ANG II infusion, were completely normalized with AZL cotreatment; on day 10, the mean BP measured over 24 hour of the ANG II group was 121 ± 5 and 86 ± 3 mm Hg in the ANG II + AZL group, respectively ($P < 0.01$). Despite the increased BP with ANG II infusion, no changes in heart rate were observed (Figure 1). The mean 24-hour BP was not different between VEH and AZL treatment groups; MBP was 92 ± 5 and 88 ± 6 mm Hg on day 10, respectively.

Normalization of ANG II-induced cardiac hypertrophy with azilsartan treatment

Infusion with ANG II for 10 days resulted in cardiac hypertrophy based on the heart/body weight ratio (Table 1 and Figure 3). Cotreatment of AZL with ANG II infusion prevented the ANG II-induced cardiac hypertrophy; the heart/body weight ratio of the cotreatment group being

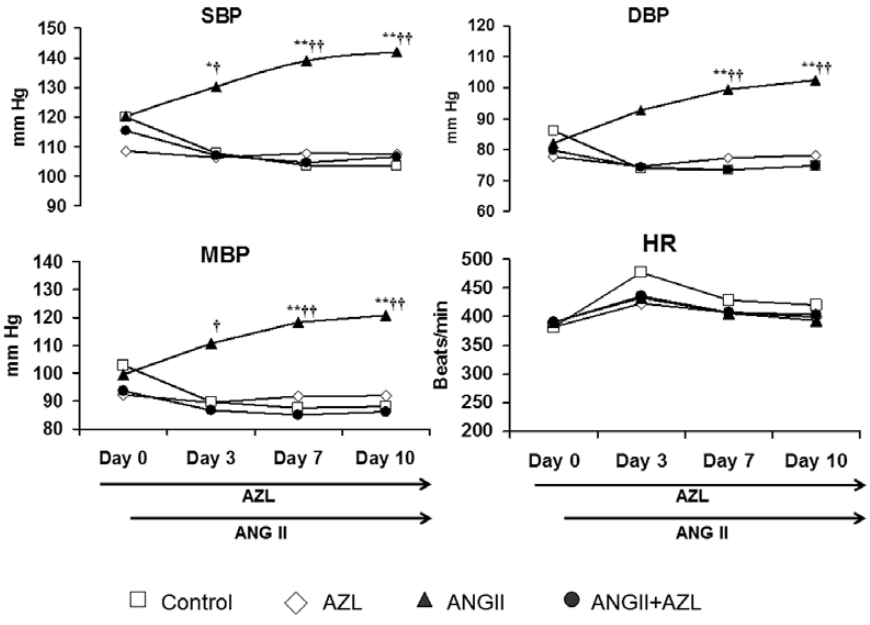

Figure 1. Effect of angiotensin II (ANG II) infusion (125 μg/kg/day; IP), AT_1R antagonism with azilsartan medoxomil (AZL; 3 mg/kg/day; PO), or ANG II and AZL cotreatment on systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), and heart rate (HR) of Sprague-Dawley rats. For clarity, the SEM is not shown on the graph. Data are expressed as mean; n = 8; *$P < 0.05$; **$P < 0.01$ vs. vehicle control on each day; †$P < 0.05$; ††$P < 0.01$ vs. ANG II + AZ on each day. Note the scale differences.

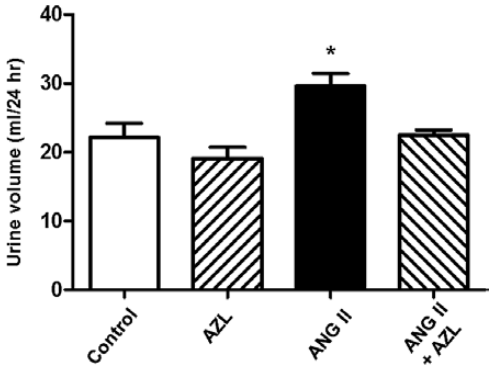

Figure 2. Urine volume after 10 days of angiotensin II (ANG II) infusion and/or azilsartan medoxomil (AZL) treatment or vehicle control. Data are expressed as mean ± SEM; n = 8; *$P < 0.05$ compared to vehicle control.

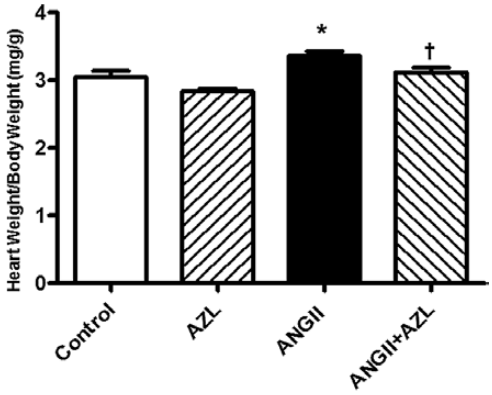

Figure 3. Heart/body weight ratio after 10 days of angiotensin II (ANG II) infusion and/or azilsartan medoxomil (AZL) treatment or vehicle control. Data are expressed as mean ± SEM; n = 8; *$P < 0.05$ compared to vehicle control; †$P < 0.05$ compared to ANG II infusion.

3.14 ± 0.09 compared to 3.04 ± 0.07 of VEH controls. No differences in kidney/body weight ratio were observed among the groups (Table 1).

Plasma ANG-(1–7) levels and urine volume after 10 days of ANG II infusion and azilsartan treatment

Plasma ANG-(1–7) levels were increased by 2- to 3-fold, compared to the VEH control group, with either treatment with AZL alone or AZL treatment together with ANG II infusion (Figure 4).

Urinary NO levels after 10 days of ANG II infusion and azilsartan treatment

Urinary nitrate/nitrite levels were measured as an index of NO levels. There were no apparent differences in NO levels seen between the treatment groups: NO (nitrate/nitrite μm/24 hour) of the VEH-treated rats were 602.10 ± 74.98 and 538.36 ± 112.76 after AZL treatment and 576.34 ± 81.02 after ANG II alone and 468.36 ± 72.55 following ANG II + AZL treatment, respectively.

Preglomerular microvessel levels of 20-HETE were attenuated by azilsartan

Following 10 days of AZL treatment, preglomerular microvessels were microdissected and lipids were extracted and analyzed by liquid chromatography–mass spectrometry. The microvascular EET/DHET levels (ng/mg protein/15min) of the VEH-treated rats were 9.1 ± 3.3 and 14.1 ± 4.0 after AZL treatment, respectively. Although not significantly increasing EET/DHET levels, AZL treatment increased the EET/DHET ratio from 0.36 ± 0.06 of the VEH-treated rats to 0.81 ± 0.15 of AZL-treated rats ($P < 0.05$). Conversely, 20-HETE levels (ng/mg protein/15 min) were significantly reduced from 1.77 ± 0.1 of the VEH-treated rats to 0.87 ± 0.14 in the vessels of AZL-treated rats ($P < 0.05$).

Histopathological effects of 10 days of ANG II infusion and azilsartan treatment

There were no histological differences observed in renal pathology between the VEH control and AZL treatment groups (Figure 5A,B). In this study, in which the animals received a normal salt (0.4% NaCl) diet, only moderate renal damage was observed in the ANG II-infused hypertensive group (Figure 5D,E,F). The damage presented mainly as vascular injury, with moderate eccentric intimal thickening of microvessels, occasional fibrinoid deposition in vessels, and presence of erythrocytes in tubules. High power magnification (100×) of sections from the ANG II-infused hypertensive group showed arterioles with extravasated and fragmented erythrocytes in swollen vessel walls, consistent

Table 1. Body and organ weights on day 10 of the study

Weight	Control	AZL	ANG II	ANG II + AZL
Body (g)	341.52 ± 6.94	328.80 ± 7.98	355.18 ± 6.31	341.69 ± 6.37
Left kidney (g)	1.202 ± 0.036	1.173 ± 0.039	1.263 ± 0.031	1.232 ± 0.035
Right kidney (g)	1.225 ± 0.042	1.219 ± 0.065	1.257 ± 0.027	1.289 ± 0.052
Heart (g)	1.039 ± 0.036	0.931 ± 0.028*	1.194 ± 0.043	1.064 ± 0.046
Kidney/body ratio (mg/g)	7.119 ± 0.218	7.213 ± 0.208	7.268 ± 0.222	7.028 ± 0.046
Heart/body ratio (mg/g)	3.044 ± 0.091	2.833 ± 0.047	3.363 ± 0.065*	3.114 ± 0.074†

Abbreviations: ANG, angiotensin; AZL, azilsartan.
Data are expressed as mean ± SEM; n = 8; *$P < 0.05$ compared to vehicle control; †$P < 0.05$ compared to ANG II.

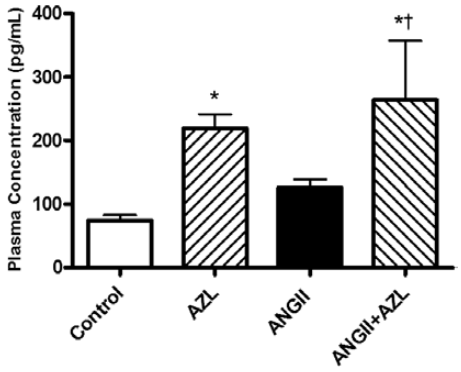

Figure 4. Plasma angiotensin (ANG)-(1–7) levels after 10 days of ANG II infusion and azilsartan medoxomil (AZL) treatment. Data are expressed as mean ± SEM; n = 8; *$P < 0.05$ compared to vehicle control; †$P < 0.05$ compared to ANG II infusion.

with thrombotic microangiopathy. In the ANG II infusion and AZL cotreatment group (Figure 5C), the degree of injury was attenuated by AT_1 receptor blockade; however, there was still some evidence of mild arteriolar hypertrophy (arrow).

DISCUSSION

This is the first report showing that in an established model of ANG II-induced hypertension, treatment with AZL results in enhanced circulating levels of the vasodilator and antihypertrophic peptide, ANG-(1–7). An additional novel finding, in this established model of ANG II-induced hypertension, is that treatment with AZL increased the EET/DHT ratio and decreased 20-HETE levels. AZL treatment completely normalized the BP elevation and as seen in other studies and prevented cardiac hypertrophy and attenuated renal damage.

Treatment with AZL increased plasma ANG-(1–7) levels comparably (about 2- to 3-fold) in both normotensive and ANG II-infused hypertensive rats. Although most, if not all, of the antihypertensive effects of AZL might simply be mediated by its ability to block AT_1 receptors, the ability of AZL to increase ANG-(1–7) levels may have contributed to the complete prevention of BP elevation in the face of continuous and prolonged ANG II infusion. ANG-(1–7) signals through the G protein-coupled *Mas* receptor and opposes not only the vasoconstrictor but also the growth-promoting actions of ANG II.[10,28,29] In the present study, 10 days of ANG II infusion resulted in cardiac hypertrophy and this growth-promoting effect was fully prevented by AZL cotreatment as reflected in the heart-to-body weight ratio. In addition, although AZL treatment alone did not significantly decrease the heart-to-body weight ratio, it significantly reduced absolute heart weight compared with VEH-treated rats. Interestingly, AZL treatment effects were specific to the heart as kidney weight and the kidney-to-body weight ratio were unaffected by ANG II and/or AZL treatment. A recent report using transgenic mice carrying the human renin and angiotensinogen genes (hRN/hANG-Tg) has shown that the BP-lowering effect of AZL was more marked than that of olmesartan.[30] The lower renal ACE2 mRNA expression seen in the hRN/hANG-Tg mice compared to control WT mice was attenuated by AZL, but not by olmesartan. These

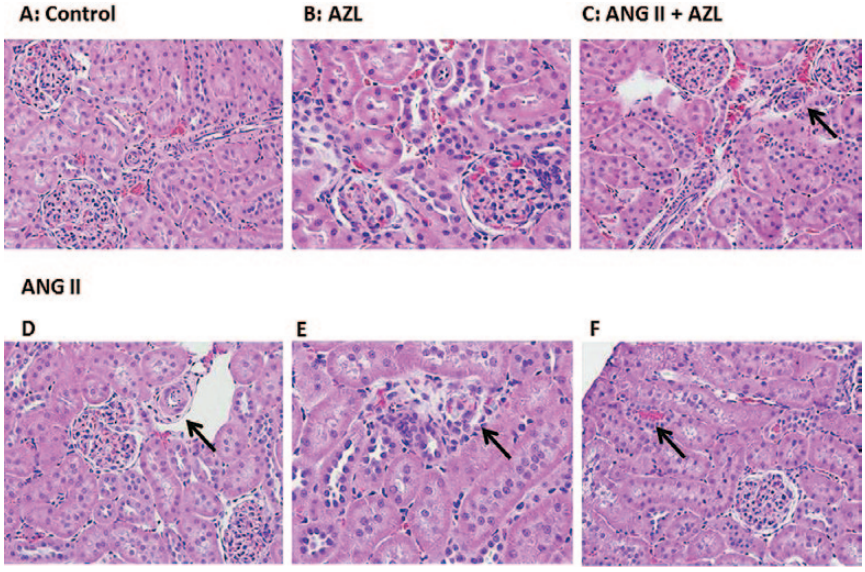

Figure 5. Representative hematoxylin and eosin stained images of kidney sections obtained on day 10 of Sprague-Dawley control animals (**A**), azilsartan medoxomil (AZL) treatment (**B**), angiotensin II (ANG II) infusion + AZL treatment (**C**), and ANG II infusion (**D**–**F**). Original magnifications ×100 on all except (**E**) ×50. Panels **A** and **B** show no significant pathology, while panel **C** shows mild arteriolar hypertrophy in a few profiles (arrow). Animals infused with ANG II alone show significant microvascular pathology as shown in panels **D** and **E**. Panel **D** shows an arteriole with eccentric intimal thickening (arrow), while panel **E** reveals a markedly swollen arteriole with fragmented extravasated erythrocytes in the vessel wall consistent with glomerular damage and thrombotic microangiopathy as seen in malignant nephrosclerosis. Please also note that adjacent tubular profiles in panels **D** and **E** showing ischemic changes characterized by epithelial swelling and attenuated brush borders. Fresh erythrocytes are also seen in some of the tubular lumina in the ANG II-infused animals (**E**), suggesting hematuria most likely due to glomerular injury.

results suggest that the effects of AZL may involve activation of the ACE2/Ang-(1–7)/*Mas* axis in addition to AT_1 receptor blockade. Although AZL increased ANG-(1–7) levels in our study, all of the cardiac and renal protective effects observed in this study might simply have been due to the effects of the drug on BP.

In one study, losartan treatment in normotensive Sprague-Dawley rats and spontaneously hypertensive rats resulted in a rise in urinary ANG-(1–7) levels.[25] In contrast, AT_1 receptor blockade had no effect on the urinary excretion of ANG-(1–7) levels in high renin, (mRen-2)27 transgenic hypertensive rats. The authors in this latter study suggested that the development of hypertension may be due a diminished capacity to convert the high ANG II levels to ANG-(1–7).[26,31] In human essential hypertensive patients, neither losartan nor eprosartan increased plasma ANG-(1–7) levels.[32] However, in agreement with the present study, losartan treatment of Lewis rats resulted in increased circulating levels of ANG-(1–7), which was associated with no change in the renal expression of *Mas* receptors. In spontaneously hypertensive rats, plasma ANG-(1–7) levels were increased with losartan treatment and infusion of an ANG-(1–7) antibody resulted in elevated MBP, whereas infusion of ANG-(1–7) in spontaneously hypertensive rats resulted in a reduction in SBP and a diuresis and natriuresis.[33,34] Recently, AZL treatment was compared to that of ANG-(1–7) administration on vascular injury using wild-type, *Mas*-knockout, and AT_2 receptor knockout mice. Treatment with AZL or ANG-(1–7) attenuated neointimal area and vascular smooth muscle cell proliferation.[35] These inhibitory effects of AZL and ANG-(1–7) were less marked in *Mas*-knockout mice and AT_2 receptor knockout mice compared with wild-type mice, suggesting that blockade of the AT_1 receptors by AZL could enhance the activities of the ACE2/ANG-(1–7)/*Mas* axis and ACE2/ANG-(1–7)/AT_2 receptor axis. In an *in vitro* study using aortic endothelial cells, AZL exhibited greater antiproliferative activity than valsartan, effects that were also observed in cells lacking AT_1 receptors. These findings suggest that AZL can function as a pleiotropic ARB with potentially beneficial effects through actions that could involve more than just blockade of AT_1 receptor and/or reduction in BP.[36]

In agreement with other studies,[37] we found that the levels of AT_2 receptor mRNA expression in the kidney cortex and medulla were undetectable and therefore we could not evaluate treatment effects. We also found no difference in urinary NO production among VEH, AZL, ANG II, or ANG II + AZL treatment groups. As NO formation in response to ANG II may depend on AT_2 receptor stimulation, this may explain why we did not observe an increase in NO levels with AZL treatment. Thus, although AZL increased ANG-(1–7) in our study, it is unlikely that NO contributed to the BP-lowering effects.

The RAS in the brain is not only a potent regulator of BP but acts as a powerful dypsogenic stimulus, via activation of AT_1 receptors. Circulating ANG II interacts with central nervous structures involved in hypovolemic thirst and BP control.[38,39] In our study, AZL cotreatment during ANG II infusion not only normalized BP but also reversed the polyuria associated with ANG II infusion, which most likely reflects a decreased dypsogenic action compared with ANG II infusion alone. Whether this is a direct effect of AZL to antagonize central AT_1 receptor activation or some other action such as stimulating ANG-(1–7) levels is unknown.

The reduction in microvascular 20-HETE with AZL is consistent with the findings that ANG II infusion increases microvascular 20-HETE levels and 20-HETE induces endothelial ACE.[18,20] 20-HETE has been shown to have prohypertensive and proinflammatory actions including inhibition of BK^+_{Ca} channel activity, uncoupling of eNOS, increasing myogenic tone, oxidative stress, vascular smooth muscle proliferation, and NF-κB activation.[16,18] In addition, we found that AZL increased the EET/DHET ratio indicative of a favorable effect on CYP metabolism of AA, which presumably may also contribute to the antihypertensive and beneficial renal and cardiac antihypertrophic actions of AZL.

Although, renal hypertrophy was absent in the ANG II-infused hypertensive group, this group still showed significant microvascular pathology. This presented as eccentric intimal thickening of microvessels, glomerular damage, and the development of thrombotic microangiopathy as seen in malignant nephrosclerosis. Despite the complete normalization of BP in the ANG II infusion + AZL group, there was still some evidence of mild arteriolar hypertrophy; therefore, AT_1 receptor blockade with AZL resulted in an attenuation of the degree of renal injury induced by ANG II-dependent hypertension. These data are consistent with recent reports that AZL reduces renal damage in obese spontaneously hypertensive rats and in Zucker diabetic fatty rats.[40,41] ANG-(1–7) has been shown to inhibit proliferation of vascular smooth muscle cells, vascular remodeling, and to oppose the mitogenic effects of ANG II.[42,43] ANG-(1–7) also inhibits epidermal growth factor receptor transactivation via a *Mas* receptor/Src-dependent pathway and thereby lessen vascular dysfunction.[44] In other studies, *Mas* receptor antagonism has been found to intensify the production of end-organ damage.[25] A limitation of our study was that the contribution of ANG-(1–7)/*Mas* pathway and/or EETs to the vascular protective effects of AZL was not examined and requires further investigation.

In summary, we have shown that in a model of ANG II-induced hypertension, AZL, a new AT_1 receptor antagonist prevented BP elevation and cardiac hypertrophy, attenuated renal damage and markedly stimulated circulating ANG-(1–7) levels, as well as, increasing the EET/DHET ratio and diminishing 20-HETE levels. The RAS plays an important role in the pathophysiology of cardiovascular and kidney diseases by altering BP and fluid and electrolyte balance. As ANG-(1–7) is considered to counterbalance the vasoconstrictor/proliferative effects of ANG II by acting as a physiological antagonist of ANG II-mediated intracellular signaling pathways, the counter-regulatory axis of the RAS system by increasing ANG-(1–7) with AZL may emerge as a novel antihypertensive therapeutic mechanism resulting from AT_1 receptor antagonism. The increased EET/DHET ratio and diminished 20-HETE levels suggest that alterations in CYP metabolism of AA may also contribute to the antihypertensive and antihypertrophic actions of AZL.

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DISCLOSURE

The authors have no conflict of interest to declare.

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