We investigated whether prevention of cardiac and vascular remodeling associated with inhibition of angiotensin II is independent of the blood pressure (BP)–lowering action of angiotensin II type 1 (AT1) receptor blockade. Spontaneously hypertensive rats, 8 weeks old, were treated with olmesartan, atenolol, or vehicle in their drinking water for 56 days. At the end of each treatment, arterial pressure and heart rate were measured, the ratio of heart weight to body weight was calculated, collagen deposition in the heart was determined histochemically using picrosirius red staining, and wall-to-lumen ratio in isolated mesenteric arteries was measured by a videographic approach. At 3 weeks after the initiation of treatment, rats medicated with olmesartan showed lower values of systolic BP compared with rats given atenolol or vehicle, whereas no difference in directly measured BP were observed at the end of study in anesthetized rats given olmesartan or atenolol. Rats given atenolol showed sustained bradycardia, whereas cardiac hypertrophy and collagen deposition was prevented only in spontaneously hypertensive rats given olmesartan. Olmesartan or atenolol reduced arteriolar wall-to-lumen ratio (olmesartan: 11.5 ± 0.4%; atenolol: 13.3 ± 0.6%; vehicle: 18.4% ± 1.1); however, this effect was greatest in rats medicated with the angiotensin II type 1 antagonist. Although control of BP is a factor in the prevention of cardiac and vascular hypertrophy, our studies suggest that blockade of angiotensin II receptors may attenuate the structural changes in the heart and blood vessels of hypertensive animals independent of a reduction in BP. Am J Hypertens 2005;18:922–929 © 2005 American Journal of Hypertension, Ltd.

Key Words: Atenolol, cardiac hypertrophy, hypertension, olmesartan, vascular remodeling.

In this study, we evaluated whether olmesartan, a new AT1 antagonist, is superior to atenolol in preventing hypertension-related progression of endothelial dysfunction in mesenteric resistance vessels and collagen deposition in the hearts of spontaneously hypertensive rats (SHR).

Methods
Experimental Protocol
A total of 24 male SHR, 6 weeks old, were obtained from Charles River Laboratories (Wilmington, MA). At 8 weeks of age, they were assigned randomly to one of three treatment groups: a) olmesartan (RNH-6270; Sankyo Pharmaceutical Company, Tokyo, Japan, 10 mg/kg BW/day); b) atenolol (Sigma, St. Louis, MO, 10 mg/kg BW/day); c) vehicle. The rats were housed in stainless steel wire mesh cages, with free access to food and water. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Wake Forest University School of Medicine.


From the Hypertension and Vascular Disease Center (HY, DBA, K.BB, RDS, CMF), Wake Forest University School of Medicine, Winston-Salem, North Carolina; and Clinical Research Institute of Montreal (ELS), Montreal, Quebec, Canada.

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Address correspondence and reprint requests to Dr. Carlos M. Ferrari, The Hypertension and Vascular Disease Center, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157; e-mail: cferrari@wfubmc.edu

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day); or c) vehicle (tap water). During the experiments, rats were housed individually under a 12-h light/dark cycle in an AAALAC-approved facility and had free access to food and drinking water. Olmesartan and atenolol, dissolved in 0.1% NaHCO₃ + KHCO₃ solution and distilled water, respectively, were given to the rats in the drinking water. The amount of drug ingested by rats was adjusted daily based on their water intake during the preceding 24 h. The Animal Care and Use Committee of Wake Forest University School of Medicine approved this study in compliance with guidelines by the National Institutes of Health. 

Tail-cuff systolic BP (Narco Bio-systems, Houston, TX) were measured at regular intervals for 3 days before and weekly during the 8 weeks after initiation of the treatment period. Heart rate was derived from the pulse waves of tail-cuff BP. At the end of the treatment period, rats were weighed and anesthetized with Inactin (Sigma, St. Louis, MO; 100 mg/kg BW given intraperitoneally). A plastic catheter (PE-50 Clay Adams; Becton Dickinson, Sparks, MD) was inserted into a carotid artery for direct measurements of arterial BP and heart rate (Biopac Systems, Goleta, CA). After collection of arterial blood from a plastic catheter, the rats were killed by intravenous injection of 75 mg/kg BW of pentobarbital sodium (Butler, Columbus, OH). A segment of the proximal jejunum nourished by branches of the main mesenteric artery was excised and placed in cold (4°C) physiologic salt solution with the following composition (in mmol/L): KCl 4.8, CaCl₂ 2.0, KH₂PO₄ 1.2, MgSO₄ 1.2, dextrose 11, NaCl 118, and NaHCO₃ 25. The heart was removed and weighed for the determination of heart weight to body weight ratio; it was then placed in 4% formalin solution.

Measurement of Small Mesenteric Artery Reactivity

Mesenteric arteries with an outer diameter of approximately 300 µm were identified and dissected with the aid of a dissecting microscope. Isolated arterial segments with a length of 2 to 3 mm were transferred to an arteriograph chamber (Living System Instrumentation, Burlington, VT). The artery segment was cannulated at each end and maintained at an intraluminal pressure of 60 mm Hg. Only leak-free preparations that maintained a stable intraluminal pressure were studied. Pre-warmed buffer (37° C) equilibrated with 21% O₂: 5% CO₂: 74% N₂ (pH 7.4) was circulated through the vessel chamber at a rate of 38 mL/min. The chamber was set on the stage of an inverted microscope with a video camera attached to the viewing tube. The vessel image was projected on computer and television monitors, and measurement of external diameter (ED), lumen diameter (LD), and intraluminal pressure were made using SoftEdge software (IonOptix Corp., Milton, MA).

After a 30-min equilibration period, ED and LD were measured, and the wall width (WW) was expressed as (ED − LD)/2. Wall to lumen ratio (W/L) was expressed as (WW/LD) × 100 and media cross-sectional area (MCSA) was calculated as (π/4) × (ED² − LD²). Continuous measurements of LD were made after determinations of vessel dimensions. To evaluate vascular reactivity, ascending concentrations of phenylephrine (10⁻¹⁰ to 10⁻⁵ mol/L; Sigma, St. Louis, MO) were applied to the abluminal surface of the isolated perfused vessel. Vessels were exposed to each concentration for 5 min before the next concentration was added, and the mean LD was recorded during each 5-min period. Thereafter, acetylcholine (10⁻⁵ mol/L; Sigma, St. Louis, MO) was added to each vessel to establish the viability of vascular endothelium. After addition of acetylcholine, the vessel was washed and allowed to equilibrate for 30 min before the next step. To evaluate vascular compliance, we determined LD during step-wise increases in transmural pressure of 20, 40, 60, 80, and 100 mm Hg. Incremental vessel distensibility was calculated as the fractional change in lumen diameter (∆LD/LD) per change in intraluminal pressure (ΔP) according to Intengan et al. 12 All drugs were added to a buffer reservoir and the buffer was recirculated. Dosages were expressed as the final cumulative molar concentration in the buffer solution. Vessels were exposed to each intraluminal pressure for 3 min before the next exposure to increase in perfusion pressure, and the maximal LD recorded during each 3-min period.

Biochemistry

Plasma concentrations of Ang I, Ang II, and Ang-(1-7) were determined by radioimmunoassay from blood collected into chilled tubes containing a mixture of 25 mM ethylenediaminetetraacetate (Sigma, St. Louis, MO), 0.44 mM L,1,2O-orthophenanthroline monohydrate, 1 mM Na⁺ para chloromercuribenzoate, and 3 mM of rat renin inhibitor: acetyl-His-Pro-Phe-Val-Statine-Leu-Phe (WFLM), as described elsewhere. 13

Histologic Evaluation of Collagen Deposition in the Heart

Heart tissue was embedded in paraffin, and 5-µm sections were transferred to subbed slides and deparaffinized by sequential washes with xylene, 100% ethanol, 95% ethanol, 75% ethanol, and double-distilled water. Tissue sections were stained with picrosirius red (0.1% solution in saturated aqueous picric acid; Sigma Chemical), examined under a light microscope, and photographed with a Zeiss AxioCam digital camera and AxioVision software (Zeiss, Thornwood, NY). The digitized images at 20 x magnifications were saved as JPEG files (1300 × 1030 pixels), and collagen deposition was determined as the number of pixels staining for picrosirius red using Photoshop version 7.0 (Adobe, San Jose, CA) according to Averill et al. 14 The collagen volume fraction was determined in left ventricular tissue.
Statistical Analysis

All data are expressed as means ± 1 SEM. Comparisons among treatment groups were analyzed by analysis of variance (ANOVA), and post hoc multiple comparisons were made by unpaired t tests with appropriate correction of the significance level for multiple comparisons. The statistical analyses for tail-cuff systolic BP and heart rate were done using two-way ANOVA with repeated measure for treatment. For dose-response studies in isolated mesenteric vessels (e.g., PE or transmural pressure), a two-way ANOVA was applied to the data where the main effects were study group (vehicle, olmesartan, or atenolol) and vessel manipulation (PE or transmural pressure). Statistical significance was set to a P value < 0.05.

Results

The 8-week treatment with either olmesartan or atenolol was associated with significant reductions in tail-cuff systolic BP that were significantly greater in animals randomized to olmesartan treatment (Fig. 1A). The decrease in systolic BP found in animals medicated with atenolol was associated with significant bradycardia, whereas heart rate did not change in animals randomized to olmesartan (Fig. 1B). Although tail-cuff systolic BP were lower in the olmesartan group between weeks 3 and 8 of the treatment period, direct measurements of arterial pressure at week 8 in anesthetized rats revealed no significant differences in systolic, diastolic, and mean arterial pressures between animals medicated with atenolol or olmesartan (Table 1).

However, differences in heart rate between the two treatments groups were still present (Table 1). Cardiac hypertrophy, measured by the ratio of heart weight to body weight, decreased significantly only in SHR medicated with olmesartan (Table 1).

Figure 2 depicts the changes in small mesenteric artery vessel dimensions produced by 8-week treatment with vehicle, atenolol, or olmesartan. Atenolol treatment was associated with significant decreases in WW and W/L ratios but no changes in ED, LD, or MCSA. In contrast, olmesartan-treated animals showed significant increases in LD and significant decreases WW and W/L ratios when compared with vehicle-treated SHR. In addition, the reduction in W/L ratio was significantly (P < .05) greater in olmesartan-treated compared with atenolol-treated SHR.

The vasoconstrictor responses to cumulative doses of phenylephrine applied to the bathing solution outside of the vessel segments were similar in animals medicated with vehicle, atenolol, or olmesartan (Fig. 3). Likewise, there were no significant differences in the vasodilator responses induced by acetylcholine among the three treatment groups (Fig. 3).

Figure 4A illustrates the change in LD in mesenteric arteries exposed to intraluminal pressures ranging from 20 mm Hg to 100 mm Hg. At intraluminal pressures between 40 mm Hg and 100 mm Hg, the LD of olmesartan-treated SHR were significantly greater than LD of vehicle-treated SHR. In contrast, the increase in LD of atenolol-treated SHR did not differ from those obtained in vehicle-treated SHR throughout the range of transmural pressures. Furthermore, vascular distensibility at intraluminal pressures <60 mm Hg was highest in olmesartan-treated SHR compared with vehicle-treated rats (Fig. 4B), whereas increased vascular distensibility of atenolol-treated SHR was greater than vehicle-treated SHR only at an intraluminal pressure of 60 mm Hg.

At 56 days after starting treatment with olmesartan, plasma concentrations of Ang I, Ang II, and Ang-(1-7) were higher in SHR medicated with olmesartan but were not changed in rats given atenolol (Fig. 5). The left ventricular collagen volume fraction was significantly reduced in the olmesartan and atenolol groups compared with the vehicle group (Fig. 6), although the ratio of heart weight to body weight was decreased only in olmesartan-treated SHR (Table 1).
Discussion

The importance of the renin–angiotensin system (RAS) in the pathogenesis of hypertension is underscored by the therapeutic effectiveness of either angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB) in subjects with hypertension. Likewise, ACE inhibitors and ARB are similarly effective in reducing BP in animal models of genetic and acquired hypertension. The predominant mode of action for ACE inhibitors and ARB appears to reside in the ability of these agents to prevent a multiplicity of effects attributed to Ang II including proliferation of smooth muscle, activation of proinflammatory factors, and impairment of vascular endothelial function.3

In patients with mild hypertension, losartan corrected the altered structure and endothelial dysfunction of resistance arteries after 1 year of treatment, whereas atenolol had no effect, in spite of similar reductions of BP.15 Brosnan et al16 have reported that irbesartan lowered superoxide levels, increased availability of nitric oxide, and improved endothelial dysfunction in stroke-prone SHR. Furthermore, treatment with ARB reduced the levels of inflammatory markers in normotensive patients with stable coronary artery disease,17 and may prevent atherogenesis, plaque instability, and thrombosis by promoting fibrinolysis.18

In keeping with findings obtained elsewhere, the BP lowering effect of olmesartan, a selective AT1 receptor antagonist, was not accompanied by changes in heart rate, whereas olmesartan or atenolol produced comparable reductions in mean arterial pressure. Although the antihypertensive effect of atenolol, as determined by weekly measures of tail-cuff systolic BP, was less than that found

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle-Treated (n = 8)</th>
<th>Atenolol-Treated (n = 8)</th>
<th>Olmesartan-Treated (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>339 ± 6</td>
<td>330 ± 4</td>
<td>349 ± 9</td>
</tr>
<tr>
<td>Heart weight (mg)</td>
<td>1075 ± 29</td>
<td>1000 ± 18</td>
<td>1006 ± 27</td>
</tr>
<tr>
<td>Heart weight/body weight ratio (mg/g)</td>
<td>3.17 ± 0.07</td>
<td>3.03 ± 0.03</td>
<td>2.88 ± 0.05*†</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>201 ± 6</td>
<td>168 ± 8*</td>
<td>155 ± 5*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>149 ± 6</td>
<td>126 ± 7*</td>
<td>119 ± 6*</td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>173 ± 6</td>
<td>146 ± 7*</td>
<td>136 ± 6*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>383 ± 8</td>
<td>329 ± 10†</td>
<td>391 ± 12</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

* P < 0.05 versus vehicle-treated rats; † P < 0.05 versus atenolol-treated rats; ‡ P < 0.05 versus vehicle-treated and olmesartan-treated rats.
in animals given olmesartan, no significant differences in mean arterial pressure were found between SHR given atenolol or olmesartan at the end of the study. The absence of differences in directly recorded BP at the end of the study was associated with no effect of atenolol on the degree of cardiac hypertrophy when compared with vehicle or olmesartan medicated SHR. The differential outcome of the two measures might be related to the fact that the tail-cuff method records systolic BP in the rat’s tail, whereas in the direct measure both systolic and mean arterial pressure were recorded at the level of the aortic arch. Alternatively, differences in the two variables might also be influenced by having made these determinations in rats with and without anesthesia.

Hemodynamic changes induced by either olmesartan or atenolol treatment resulted in comparable improvements in vascular resistance in small mesenteric arteries, both in terms of significant decreases in wall width and wall-to-lumen ratios. In contrast, only olmesartan treatment was associated with a significant increase in the LD of small resistance vessels. These results suggest that BP control has an important role in determining vascular remodeling in SHR, although the same mechanism may be less dominant in terms of cardiac remodeling. In hypertension, vascular remodeling involves eutrophic and hypertrophic remodeling. In eutrophic remodeling, wall material is rearranged around a reduced lumen without evidence of net growth, whereas in hypertrophic remodeling, an increase in vessel media cross section encroaches on the vessel lumen. The differing effects of atenolol versus olmesartan on LD indicate that olmesartan has a greater effect in preventing eutrophic remodeling.

In the current study, vascular reactivity, measured as the vasoconstrictor and vasodilator responses to several doses of phenylephrine and acetylcholine, respectively, were not different among SHR treated with vehicle, atenolol, or olmesartan. In keeping with these findings, a differential effect of hypertension on vascular structural remodeling and endothelial dysfunction has been documented in experimental models of hypertension as well as in subjects with essential hypertension. On the other hand, changes in LD and vascular distensibility of small resistance arteries were significantly greater only in SHR treated with olmesartan. These data suggest that blockade of Ang II receptors has a more pronounced effect in the normalization of vessel wall dynamics and structure that what can be achieved by the protective effect of BP control.

Reflecting the greater effect of olmesartan on vascular structure, vascular compliance of resistance arteries was greater than that in atenolol treated SHR. These results suggest that blockade of AT1 receptors is more effective than atenolol in preventing or attenuating hypertensive vascular injury and remodeling of small resistance arteries. Differences in the ability of the two agents to alter the course of vessel remodeling were paralleled by the greater

**FIG. 4.** Lumen diameter–intraluminal pressure curves (A) and incremental distensibility–intraluminal pressure curves (B) in mesenteric resistance arteries from spontaneously hypertensive rats randomized to olmesartan (○), atenolol (●), or vehicle (★) for 8 weeks of treatment. Values are means ± SEM. *P < 0.05 versus vehicle group.

**FIG. 5.** Olmesartan caused a significant increase in plasma concentration of angiotensins, ie, Ang I, Ang II, and Ang-(1-7), at completion of the treatment period (day 56). Values are means ± SEM for SHR given vehicle (white bar), atenolol (gray bar), or olmesartan (black bar).
effect of olmesartan in reversing cardiac hypertrophy. These data are in agreement with previously reported studies in experimental hypertension\textsuperscript{12,21,22} and the recent demonstration that reversal of cardiac hypertrophy in essential hypertensive patients medicated with losartan during a 5-year period was significantly greater than that found in subjects assigned to the atenolol arm of the study.\textsuperscript{23}

Changes in sympathetic nervous system (SNS) activity may alter vascular remodeling as vascular smooth muscle proliferation is stimulated by norepinephrine in vitro, and this effect is inhibited by atenolol.\textsuperscript{24} Although it remains controversial whether $\beta$-adrenergic blockade stimulates a compensatory increase in $\alpha$-adrenergic drive, Burns et al.\textsuperscript{25} reported that the antihypertensive effect of atenolol did not increase peripheral sympathetic nervous system activity and vascular resistance. On the other hand, peripheral or central blockade of AT\textsubscript{1} receptors with losartan prevented the increase of BP and sympathetic nervous system activity induced by intra-renal phenol.\textsuperscript{26} In addition, Uresin et al.\textsuperscript{27} found that losartan did not increase plasma catecholamine concentrations, although it attenuated sympathetic nervous system overactivity induced by stressful stimuli. One might also speculate that in the presence of AT\textsubscript{1} blockade Ang II acts at Ang II type 2 receptors to prevent vascular remodeling.\textsuperscript{3} However, we have shown that Ang II type 2 blockade has no effect on the hemodynamic and neurohormonal response to administration of losartan in rats.\textsuperscript{28,29}

A limitation of the present study is that tail-cuff systolic BP between weeks 3 and 8 of the treatment period were lower in the olmesartan-treated animals compared with those given atenolol. However, differences in BP were not found at the end of the study in the anesthetized animals. Moreover, left ventricular hypertrophy was still present in rats randomized to atenolol treatment, whereas cardiac hypertrophy was reduced in rats medicated with olmesartan. These data suggest, but obviously do not prove, that blockade of AT\textsubscript{1} receptors prevents vascular remodeling by a mechanism that is only in part is directly related to

**FIG. 6.** Photomicrographs of cardiac tissue for SHR given vehicle (A), olmesartan (B), or atenolol (C) for 8 weeks. Picrosirius red staining of heart tissue demonstrated that vehicle-treated SHR had substantially greater collagen deposition when compared with olmesartan-treated or atenolol-treated SHR (D). Values are means ± SEM. *$P < 0.05$ versus vehicle group.
arterial pressure. This interpretation is in keeping with studies in human subjects and animals showing a superior effect of AT\(_1\) blockade compared with that achieved with atenolol in attenuating altered vascular structure and cardiac function.\(^{3,15,21,30,31}\) The mechanism by which atenolol may affect vascular remodeling may be related to decreased BP and partial interruption of the renin angiotensin system, as Malmqvist et al\(^{31}\) showed a small but statistical significant effect of atenolol on plasma concentrations of Ang II in human subjects. Although, in our experiments, plasma Ang II levels were not different from those observed with vehicle in SHR given atenolol, it is necessary to point out that the reduction in plasma Ang II observed in the study reported by Malmqvist et al\(^{31}\) averaged \(-0.2\) pmol/L at week 48 of treatment. The small effect of atenolol on plasma Ang II levels in this study does not suggest an important role of Ang II in mediating a nonhemodynamic effect of the drug in preventing cardiac hypertrophy.

In conclusion, our data demonstrated that BP reduction by olmesartan or atenolol was associated with attenuation of vascular hypertrophy and remodeling. However, olmesartan more effectively ameliorated the structural effects of hypertension in the heart and mesenteric resistance vessels by a mechanism that was only partially dependent on BP control.

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References


