Effects of Losartan and Enalapril on Small Artery Structure in Hypertensive Rats

Damiano Rizzoni, Enzo Porteri, Alfonso Piccoli, Maurizio Castellano, Giorgio Bettoni, Maria Lorenza Muiesan, Giancarlo Pasini, Daniele Guelfi, Michael J. Mulvany, Enrico Agabiti Rosei

Abstract—We evaluated the effects on cardiovascular structure of the angiotensin-converting enzyme (ACE) inhibitor enalapril and of the angiotensin II receptor blocker losartan, administered either at hypotensive or nonhypotensive dosage in spontaneously hypertensive rats (SHR). SHR were treated from ages 4 to 12 weeks with low-dose (1 mg \( \cdot \) kg \( ^{-1} \) \( \cdot \) d \(^{-1} \)) enalapril, low-dose (0.5 mg \( \cdot \) kg \( ^{-1} \) \( \cdot \) d \(^{-1} \)) losartan, high-dose (25 mg \( \cdot \) kg \( ^{-1} \) \( \cdot \) d \(^{-1} \)) enalapril, or high-dose (15 mg \( \cdot \) kg \( ^{-1} \) \( \cdot \) d \(^{-1} \)) losartan. Untreated WKY and SHR were also studied. Rats were killed at 13 weeks of age, and the heart was weighed. Mesenteric small arteries were dissected and mounted on a micromyograph for determination of media thickness and lumen diameter. In fixed arteries, cell volume, number of cells per segment length, and number of cell layers were measured using the unbiased “disector” method. Systolic blood pressure was significantly reduced by the high doses of both drugs, but the hypotensive effect was greater with enalapril than with losartan (\( P<0.05 \)). In the high-dose enalapril and losartan groups, there were similar reductions in relative left ventricular mass, media/lumen ratio, and number of cell layers of resistance arteries; however, there were no differences in the cell volume or number of cells per segment length of resistance arteries. Low-dose enalapril did not affect systolic blood pressure or any of the structural parameters. The results show that the hypotensive effects of both losartan and enalapril were associated with outward remodeling of resistance arteries at the cellular level. The effect of losartan on resistance artery structure was equal to that of enalapril, despite the smaller hypotensive effect. (Hypertension. 1998;32:305-310.)

Key Words: losartan ■ enalapril ■ hypertrophy ■ angiotensin-converting enzyme inhibitors ■ angiotensin II ■ vascular resistance

The renin-angiotensin-aldosterone system seems to play a key role in the development of cardiac and vascular hypertrophy that is usually observed in both humans and animal models of genetic or experimental hypertension. Thus, because vascular structural alterations are importantly involved in the mechanisms that determine blood pressure, their regression is generally regarded as an important target of antihypertensive therapy. Furthermore, ACE inhibitors have proved effective in reducing not only blood pressure but also cardiac mass and structural alterations in small arteries in both humans and SHR. Whether the regression of cardiovascular alterations is due only to blood pressure reduction or is also a consequence of growth factor inhibition remains controversial.

Recently, nonpeptide selective inhibitors of AT\(_1\) receptors have been developed and introduced as antihypertensive agents. Like ACE inhibitors, these drugs may cause regression of cardiac and vascular structure in SHR, and the effects on cardiovascular structure appear to be similar. Nevertheless, there are theoretical grounds for supposing that AT\(_1\) receptor antagonists could be more effective than ACE inhibitors for structural abnormalities in both the heart and the vessels. First, selective inhibition of AT\(_1\) receptors is accompanied by an increase in circulating (and probably also in tissue) levels of angiotensin II. The raised angiotensin level will presumably cause increased stimulation of AT\(_2\) receptors, which could further inhibit smooth muscle cell growth and stimulate cellular apoptosis. Second, in the heart and the vessels, because angiotensin II may be produced through chymase-dependent pathways, thus bypassing ACE inhibition, direct inhibition of the AT\(_1\) receptor blockers may be more effective than ACE inhibitors. It is therefore possible that although the effect of the two classes of drugs on the gross morphology of the arteries is similar, there are potential differences at the cellular level.

Ledingham and Laverty recently evaluated the effects of the angiotensin II receptor antagonist valsartan on structural alterations in mesenteric small resistance arteries of New Zealand genetically hypertensive rats using a stereological method. However, the New Zealand hypertensive rats showed the presence of hypertrophic remodeling. On the other hand, in mesenteric arteries of SHR an inward eutrophic remodeling was observed; the same pattern of structural alterations may be detected in human essential hypertension.

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On this basis, we therefore decided to undertake a new investigation to compare in SHR (a rat strain in which vascular morphology is similar to that seen in hypertensive patients) the dose-dependent effect of ACE inhibitors and AT₁ receptor antagonists, not only on cardiac mass and resistance artery morphology but also on the size and number of the smooth muscle cells within the arteries, using an unbiased stereological technique. Two doses of the AT₁ receptor antagonist losartan and of the ACE inhibitor enalapril were used; the doses were chosen to provide either no effect on blood pressure (low dose) or a near normalization of blood pressure (high dose). Antihypertensive treatment was started in a prehypertensive phase to prevent the development of hypertension.

Methods

One hundred rats (82 SHR and 18 WKY) were included in the study. The animals were obtained from Charles River Laboratories (Calco, Italy). All the procedures followed were in accordance with the guidelines of our institution (Medical School, University of Brescia). The rats were housed 2 per cage in a room in which the temperature was controlled between 23°C and 25°C and a 12-hour light/dark cycle was maintained. Food and water were supplied ad libitum.

Sixteen SHR were treated with low-dose losartan (0.5 mg · kg⁻¹ · d⁻¹), 16 with high-dose losartan (15 mg · kg⁻¹ · d⁻¹), 16 with low-dose enalapril (1 mg · kg⁻¹ · d⁻¹), and 16 with high-dose enalapril (25 mg · kg⁻¹ · d⁻¹). Both drugs were administered in the drinking water from the 4th to the 12th week of age. Eighteen WKY and 18 SHR were kept untreated as controls. Rats were killed at 13 weeks of age.

Systolic blood pressure values and heart rate were measured noninvasively (tail-cuff method, IITC Life Science Instruments) in conscious rats each week.

On the day of death, the animals were weighed and then killed by decapitation. The heart was promptly dissected, dried, and weighed, and the HW/BW was calculated in all the animals; in addition, in both treated and untreated SHR, the RLVM (left ventricular weight/body weight) was calculated. At the same time, from each rat, mesenteric vessels corresponding to the second branch (about 140 to 200 μm of average diameter in relaxed conditions, 2 mm long) were obtained by dissection. The vessel segments were excised free of connective and adipose tissue, and 2 stainless steel wires of 40 μm in diameter were threaded through the lumen. This ring preparation was mounted on a microgunograph as previously described by Mulvany et al. Total time for dissection and preparation was approximately 45 minutes. Vessels were then equilibrated and relaxed for at least 30 minutes in physiological saline solution of the following composition (in mmol/L): NaCl 119, NaHCO₃ 24, KCl 4.7, KH₂PO₄ 1.18, MgSO₄ 1.17, CaCl₂ 2.5, and glucose 5.5, kept constantly at 37°C and bubbled with 5% CO₂ in O₂.

After equilibration, the microgunograph was transferred to the stage of a light microscope with immersion lens. The vessel was stretched slightly (wall tension ~0.1 N/m), and structural characteristics of the vessels were evaluated. The following parameters were measured: wall thickness, media thickness, adventitia thickness, intima thickness, internal diameter, media/lumen ratio, and media cross-sectional area. Then the normalized internal circumference L₁ was determined, as described previously by Mulvany et al., from the resting wall tension–internal circumference relation and Laplace equation (L₁ is defined as 0.9 × L₁₀₀, where L₁₀₀ is an estimate of the internal circumference that the vessel would have had in vivo when subjected to a transmural pressure of 100 mm Hg while relaxed). From L₁, the normalized internal diameter d₁ was calculated. Assuming that the cross-sectional area remains constant when the vessel is extended to L₁, the previously mentioned morphological parameters were automatically calculated in a normalized condition also. For further details, see References 24 and 25.

A “remodeling index” was calculated in untreated SHR and WKY according to the method of Heagerty et al., expanding a previous observation of Baumbach and Heistad. This index quantifies how much of the vascular structural alteration may be explained by a rearrangement of the same material around a narrowed lumen, without cell growth. The formula for calculation of remodeling index is:

$$RI = 100 \times \left( \frac{(ID_{h})^2 - 4 \times CSA / \pi}{(ID_{n})^2 - 4 \times CSA / \pi} \right)$$

where ID indicates media internal diameter (media + intima); ED, media external diameter; CSA, media cross-sectional area; n, normal subjects; h, hypertensive subjects; and RI, remodeling index.

When the micromyograph measurements were complete, the bathing solution was changed to calcium-free saline for 10 minutes to prevent a vasoconstrictive effect of the fixative. With the arteries still on the wires, the solution was changed to fixative (buffered glutaraldehyde 2%). The vessels were unmounted, washed in physiologically saline solution, preembedded in agar to maintain orientation, and finally embedded in historesin (Technovit 7100, Heraeus Kulzer). In each artery from a point approximately halfway between where the mounting wires had been, a series of three to five 3-mm (±0.05) serial sections parallel to the vessel axis were made on a precision microtome (Histostage, LKB). All sections were placed on glass slides, coded, and stained with Giemsa stain.

Unbiased estimates of smooth muscle cell number within the arteries were determined using a modified version of the disector principle described previously. In brief, two successive sections were placed under two specially equipped microscopes projecting the images of the sections side by side onto a table top at a total magnification of ×1650. The number of nuclei present in the first section, but not in the second, was counted (“downward pointing” nuclear ends). Because the time-consuming event in this procedure was to find the corresponding areas in the two sections, efficiency was greatly improved by also counting the number of nuclei present in the second section but not in the first (“upward pointing” nuclear ends). Ten areas in each vessel were marked and counted. On the assumption that each smooth muscle cell contained one and only one nucleus (in examining many thousands of cell profiles, we have never seen more than one nucleus per cell), the mean number of smooth muscle cells per unit volume media (the cell numerical density) could be calculated by dividing half the total number of nuclear ends by the total disector volume. From cell numerical density and volume fraction of media containing smooth muscle cells, the mean cell volume was calculated. Additionally, the following parameters were calculated: average nucleus length, cell length, cell cross-sectional area, number of cell layers, and number of cells per unit vessel length. The equations used for the calculation of the previously mentioned morphological parameters are reported in References 27 and 28. Morphological results from two different blood vessels in each rat were averaged to provide one mean observation per subject.

Statistical Analysis

All data are expressed as mean ± SD unless otherwise stated. One-way ANOVA and Bonferroni’s correction for multiple comparisons were used to evaluate differences among groups. A nonparametric approach (Mann-Whitney rank sum test) was adopted for those variables that were not normally distributed. Two-way ANOVA for repeated measures was used for blood pressure and heart rate.
Results

Blood Pressure and Heart Rate

Systolic blood pressure values in untreated and treated SHR and WKY from the 4th to the 13th week are reported in the Figure; systolic blood pressure values at the time of death are reported in Table 1. At 4 weeks of age, no statistically significant difference in systolic blood pressure was observed between untreated SHR and WKY. During the treatment period, systolic blood pressure was significantly higher in untreated SHR than in WKY controls (ANOVA, P<0.001). The SHR treated with high-dose losartan or enalapril showed a significant reduction in systolic blood pressure (ANOVA, P<0.001 versus untreated SHR during the treatment period);

high-dose enalapril had a greater hypotensive effect than high-dose losartan (ANOVA, P=0.012). No significant reduction in systolic blood pressure was observed in rats treated with low-dose enalapril or losartan. Heart rate during the treatment period (ANOVA P<0.001) and at the time of death (Table 1) was significantly lower in untreated WKY than in the other groups.

Cardiac Morphology

The values of heart weight, left ventricular weight, body weight, HW/BW, and RLVM are reported in Table 2. The RLVM was significantly increased in untreated SHR compared with untreated WKY, whereas a significant reduction was observed in the groups of SHR treated with high-dose losartan or enalapril. No effect was observed in rats treated with low-dose enalapril and losartan.

Vascular Morphology

Values of media thickness, wall thickness, media cross-sectional area, internal diameter, and media/lumen ratio in mesenteric small resistance arteries of SHR and WKY are reported in Table 3. Untreated SHR showed the presence of vascular structural alterations, as indicated by an increased media/lumen ratio. Treatment with high-dose losartan or enalapril induced a significant and similar reduction of media/lumen ratio, media thickness, and wall thickness in the SHR. No effect with low-dose enalapril and losartan was observed (Table 3). The remodeling index was very close to 100% in all groups of rats.

A significant correlation between media/lumen ratio, media thickness, or wall thickness and the average systolic blood pressure was found (Table 3).

TABLE 1. Systolic Arterial Pressure and Heart Rate at Time of Death

<table>
<thead>
<tr>
<th>Code</th>
<th>Groups</th>
<th>Systolic Arterial Pressure, mm Hg</th>
<th>Heart Rate, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated WKY (n=18)</td>
<td>157±15.6</td>
<td>357±25.1</td>
</tr>
<tr>
<td>2</td>
<td>Untreated SHR (n=18)</td>
<td>216±24.9</td>
<td>438±40.3</td>
</tr>
<tr>
<td>3</td>
<td>SHR low-dose losartan (n=16)</td>
<td>216±17.6</td>
<td>434±31.4</td>
</tr>
<tr>
<td>4</td>
<td>SHR high-dose losartan (n=16)</td>
<td>184±18.0</td>
<td>430±34.8</td>
</tr>
<tr>
<td>5</td>
<td>SHR low-dose enalapril (n=16)</td>
<td>218±18.4</td>
<td>412±50.5</td>
</tr>
<tr>
<td>6</td>
<td>SHR high-dose enalapril (n=16)</td>
<td>157±16.3</td>
<td>425±51.1</td>
</tr>
</tbody>
</table>

Superscript numbers refer to groups for which the values are significantly different (at least P<0.05 after Bonferroni’s correction).

TABLE 2. Body Weight and Cardiac Mass Indexes

<table>
<thead>
<tr>
<th>Code</th>
<th>Groups</th>
<th>Body Weight, g</th>
<th>Heart Weight, g</th>
<th>HW/BW</th>
<th>LWV, g</th>
<th>RLVM, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated WKY (n=18)</td>
<td>281±23</td>
<td>1.02±0.27</td>
<td>3.62±0.18</td>
<td>0.68±0.11</td>
<td>2.42±0.32</td>
</tr>
<tr>
<td>2</td>
<td>Untreated SHR (n=18)</td>
<td>276±27</td>
<td>0.99±0.08</td>
<td>3.59±0.16</td>
<td>0.73±0.07</td>
<td>2.64±0.11</td>
</tr>
<tr>
<td>3</td>
<td>SHR low-dose losartan (n=16)</td>
<td>289±22</td>
<td>1.01±0.07</td>
<td>3.58±0.09</td>
<td>0.74±0.05</td>
<td>2.61±0.15</td>
</tr>
<tr>
<td>4</td>
<td>SHR high-dose losartan (n=16)</td>
<td>280±22</td>
<td>0.86±0.07</td>
<td>3.14±0.16</td>
<td>0.62±0.05</td>
<td>2.23±0.13</td>
</tr>
<tr>
<td>5</td>
<td>SHR low-dose enalapril (n=16)</td>
<td>286±17</td>
<td>1.00±0.06</td>
<td>3.50±0.15</td>
<td>0.72±0.05</td>
<td>2.54±0.14</td>
</tr>
<tr>
<td>6</td>
<td>SHR high-dose enalapril (n=16)</td>
<td>273±23</td>
<td>0.84±0.08</td>
<td>3.13±0.18</td>
<td>0.58±0.06</td>
<td>2.15±0.12</td>
</tr>
</tbody>
</table>

LWV indicates left ventricular weight.

Superscript numbers refer to groups for which the values are significantly different (at least P<0.05 after Bonferroni’s correction).
pressure during therapy was observed when treated and untreated SHR were considered together ($r=0.57$, $r=0.56$, and $r=0.50$, respectively; $P<0.001$). The value of media/lumen ratio observed in the SHR treated with high-dose losartan was significantly below the value expected from the regression between systolic blood pressure at time of death in untreated SHR and WKY (predicted value, $0.118\pm0.006$; observed value, $0.108\pm0.015$; $P<0.05$); this was not the case for SHR treated with high-dose enalapril or with the low-dose of each drug.

**Cellular Morphology**

No significant difference in cell volume, cell length, cell cross-sectional area, or number of cells per segment length was observed among the groups (Table 4). The number of cell layers was greater in untreated SHR in comparison with WKY controls and was significantly reduced in SHR treated with high-dose losartan or enalapril. No significant difference was observed in rats treated with low-dose losartan or enalapril compared with untreated SHR. A significant correlation between the number of cell layers and the average systolic blood pressure during therapy was observed when treated and untreated SHR were considered together ($r=0.51$, $P<0.001$).

**Discussion**

The main finding of this study is the first demonstration that the hypotensive effects of both the AT$_1$ receptor antagonist losartan and the ACE inhibitor enalapril are associated with a remodeling of resistance arteries at the cellular level. In both essential hypertensive patients $^{21-29}$ and in SHR $^{1,21,24,27}$ the resistance vessels have an abnormal structure, such that the lumen is reduced and the media/lumen ratio is increased. However, smooth muscle cell volume is normal $^{27,30}$ indicating a lack of cellular hypertrophy. The possible presence of cellular hyperplasia is controversial. In fact, in small arteries from untreated SHR, $^{27}$ an increase in the number of cells per segment length was observed compared with in WKY controls, suggesting the presence of cellular hyperplasia. In essential hypertensive patients, there was no difference in the number of smooth muscle cells per small artery segment length. $^{30}$ The present results indicate that the number of smooth muscle cells in SHR small mesenteric arteries is not significantly different from that of WKY, without any evidence of significant hyperplasia. It may be noted, however, that there is a tendency to an increase in cell number, so the discrepancy with the previous work of Mulvany et al $^{27}$ may be due only to statistical variance.

Our finding that treatment of SHR with losartan or enalapril caused near normalization of mesenteric small artery media/lumen ratio is in agreement with previous studies concerning treatment of both men and animals with AT$_1$ receptor antagonists and ACE inhibitors. $^{3-5,15-17,20,31-33}$ Similar findings have also been reported in more proximal vascular districts. $^{34,37}$ The main novel finding of the present study is that the normalization in both cases is due to a rearrangement of otherwise similar cells. This is demonstrated by the measurements of cellular dimensions made using a stereological method that avoids many of the pitfalls associated with other methods. $^{36}$ These measurements showed that the cell morphology (length, cross-sectional area, volume) is not affected by any of the treatments, the only difference being a reduction in the number of cell layers. The effect of treatment is therefore an outward eutrophic remodeling of the small
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observed a reduction in the number of cell layers in rats treated with perindopril, whereas little or no effect was observed with captopril, whereas little or no effect was observed with losartan and enalapril. A similar indication was given in the study of Morton et al., in which losartan and captopril had the same effect on mesenteric small artery structure, although captopril had a greater hypotensive effect than losartan (at least when treatment was given from the 3rd to the 13th week of age).

Therefore, our data, like those of Morton et al., suggest the possibility that AT₁ receptor antagonism has a pressure-independent effect on vascular structure. This hypothesis is in keeping with the concept that the hemodynamic effect of an antihypertensive drug is only an important, but not exclusive, factor in determining small artery structure.

Our data with enalapril treatment are in agreement with those of Thybo et al.,. In that study, perindopril treatment was shown to have a dose-dependent effect on blood pressure as well as on structural parameters in different vascular beds of SHR. The present data are, however, at variance with a previous study in which a significant reduction was observed in media/lumen ratio of mesenteric small resistance arteries of SHR after treatment with low nonhypotensive doses of fosinopril. A possible explanation of these conflicting results may be an heterogeneity of the action of different ACE inhibitors, perhaps related to a different dose-dependent penetration in the cardiovascular tissues. In fact, fosinopril shares with zofenopril the highest lipid solubility among the ACE inhibitors currently available. Other possible explanations could be a peculiar effect of fosinopril, independent from ACE inhibition, or the possibility that a small reduction of systolic blood pressure, although not statistically significant, could have had a confounding role in the results obtained; however, in the previously mentioned study, the effect on vascular morphology was greater than that expected on the basis of the blood pressure reduction. In any case, the data suggest that the renin-angiotensin-aldosterone system may induce vascular structural alterations in part by a pressure-independent mechanism. Furthermore, we have observed a reduction in the number of cell layers in rats treated with high-dose losartan or enalapril, in agreement with Korsgaard et al. In their study, a significant reduction of cell layers was observed in SHR treated with captopril and perindopril, whereas little or no effect was observed with isradipine or metoprolol. This again suggests that resistance vessel structure is not pressure-dependent only.

Our study was aimed at investigating the effects of prevention of hypertension, rather than treatment, since antihypertensive therapy was started in young SHR before the development of overt hypertension. It is not known whether a later treatment, started when animals have developed established hypertension, would have similar effects.

In conclusion, our data show that both losartan and enalapril are effective in reducing cardiac mass and structural alterations in mesenteric small resistance arteries. This was achieved despite the fact that at the doses used, losartan had a smaller hypotensive effect than enalapril.

Acknowledgments

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