Enalapril and Losartan Reduced Cardiac Mass and Improved Coronary Hemodynamics in SHR

Eduardo Nunez, Kazuyoshi Hosoya, Dunko Susuc, Edward D Frohlich

Abstract Among the multiple mechanisms postulated for the increased risk of hypertensive left ventricular hypertrophy (LVH), coronary hemodynamic alterations remain a strong possibility. This study was designed to compare the effects of treatment with an ACE inhibitor (enalapril) and an angiotensin AT1 receptor antagonist (losartan) on systemic and coronary hemodynamics and to determine whether the combination of these two renin-angiotensin system (RAS) inhibitors would be as or more effective in reducing mean arterial pressure (MAP), left ventricular (LV) mass, and improving coronary hemodynamics than either regimen alone. Thus, 23 week old spontaneously hypertensive rats (SHR) were treated (12 weeks) with tap water (C), enalapril (30 mg kg⁻¹ d⁻¹), losartan (30 mg kg⁻¹ d⁻¹), or their combination (15 mg kg⁻¹ d⁻¹). Age-matched Wistar-Kyoto (WKY) rats served as normotensive controls. After 12 weeks, systemic and coronary hemodynamics were determined (15 μm radiolabeled microspheres) at baseline, during maximal treadmill exercise, and during maximal dilation (dipyridamole). Enalapril and losartan equally reduced MAP and LV mass in association with a decreased total peripheral resistance. The RAS combination reduced MAP and LV mass more than either drug alone. Resting cardiac index and coronary blood flow (CBF) per unit of LV mass did not differ among the groups. Although enalapril did not improve coronary flow reserve (CFR), it diminished minimal coronary vascular resistance (MCVR). Losartan improved both. However, the combination was more effective than either agent alone, reaching values close to normotensive WKY controls. In conclusion, these data demonstrated significantly improved maximal CBF, CFR, and MCVR in untreated SHR, but losartan alone and in combination with enalapril improved systemic and coronary hemodynamics more than enalapril alone (Hypertension. 1997;29[part 2]:519-524.)

Key Words • left ventricular hypertrophy [reversal] • angiotensin II [antagonism and inhibition] • drug synergy • losartan • enalapril • coronary circulation • spontaneously hypertensive rats

Left ventricular hypertrophy resulting from hypertension is an independent risk factor for premature cardiovascular morbidity and mortality, although LVH reversal has not yet been shown to reduce that risk. LVH has been associated with impaired coronary hemodynamics, including decreased CBF and CFR, increased resting and MCVR, and altered vasoactivity; cardiac arrhythmias, cardiac failure, and accelerated atherosclerosis of epicardial arteries. Current goals of antihypertensive therapy have been directed toward optimal control of arterial pressure, although reversal of LVH and improvement of the compromised coronary hemodynamics are the subject of intense investigation directed toward reducing LVH risk. Accordingly, this study was designed to determine whether treatment with an ACE inhibitor (enalapril), an AT1 receptor antagonist (losartan), or their combination improved coronary hemodynamics in association with reduced LV mass.

Methods

Procedures

Male SHR and normotensive WKY rats were obtained from Charles River Breeding Laboratories Inc (Wilmington, Mass) at 14 weeks of age and were maintained thereafter in accordance with our institutional animal care committee guidelines after approval of the experimental protocol. They were housed in temperature-controlled rooms on a 12-hour light/dark cycle and were fed usual rat chow and water ad libitum. At 23 weeks of age, the rats were randomly assigned to one of five groups of 16 rats each: untreated SHR-C (tap water), SHR-E (30 mg kg⁻¹ d⁻¹ by gavage), SHR-L (30 mg kg⁻¹ d⁻¹ by gavage), and SHR-E&L (5 mg kg⁻¹ d⁻¹ of each agent by gavage). The rats followed their respective treatments for 12 weeks. All rats were trained to run on a motor-driven treadmill (Columbus Instruments) at speeds ranging from 0.5 to 1.0 m/min, 0% grade, for 5 min/day for 2 to 3 days a week during the final 2 weeks of the protocol to familiarize them with treadmill running.

At 35 weeks of age, the rats were instrumented for determination of systemic and coronary hemodynamics using the reference standard microsphere method as described previously by our laboratory. Thus, under ketamine (10 mg/kg) and acepromazine (50 mg/kg) anesthesia, they were cannulated with three polyethylene catheters (PE-50) one inserted into the LV (via right carotid artery) for injection of microspheres, a second inserted into the caudal (tail) artery for arterial pressure measurement and for reference sample withdrawal, and a third inserted into a jugular vein for dipyridamole infusion. These catheters were exteriorized at the nape of the neck and filled with heparinized saline solution (200 U/mL), and the rats were returned to their nonrestrictive plastic cages to recover fully over at least 3 hours. Radiolabeled (55Co, 113Sn, and 46Sc) microspheres (DuPont) of 15-μm diameter were used for each blood flow measurement. Thus, 100,000 microspheres, suspended in 0.045 mL saline containing <0.01% Tween 80, were infused into the LV over 5 seconds, followed by 0.5 mL warm saline flush injection over 15 seconds. Before infusion, the microsphere suspension was mixed thoroughly on a touch mixer (Vortex) for 1 to 2 minutes, during the infusion, the Silastic tube containing the microsphere suspension was constantly tapped to ensure even distribution. The reference sample was withdrawn at a rate of 0.45 mL/min over 60 seconds (including 10 seconds before and 20 seconds after injection). Efficacy of microsphere-blood mixing was assessed by comparing...
blood flows to the right and left kidneys; a difference of ≥15% for these two flows was considered unacceptable, and the study was excluded. Adequate trapping of microspheres was evaluated by counting lung radioactivity, and if the percentage of blood flow to the lungs was 5% or greater (excessive AV shunt), the rat was also eliminated from the study. At the end of the study, the rat was killed with an overdose of pentobarbital, and the heart was removed immediately. After the atria was discarded, the free wall of the RV was carefully removed, leaving the LV free wall and septum as total LV weight. Further, the LV was dissected into two segments, subepicardium and subendocardium, to assess regional myocardial perfusion. These segments were carefully cleaned, blotted dry, and weighed on a precision gram balance. Ventricular weights were normalized for body weight and expressed as RV and LV mass (mg/g). A fixed segment (4.5 cm) of the descending aorta, starting at a point distal to the subclavian artery, was removed, weighed, and expressed as aortic mass index (mg mm\(^{-2}\) g\(^{-1}\)). Tissue samples, as well as the blood reference sample, were placed in plastic scintillation vials and counted for 15 minutes in a deep-well gamma scintillation spectrometer (Packard) using a multichannel analyzer.

CO and CBF were calculated from the counts per minute and the sample wet weights according to standard procedures after appropriate corrections for decay and background. Spillover correction between channels was achieved using INSTRUMENTATION SOFTWARE (Compusphere, Packard). Thus, CO and the CBF were calculated using these formulas: CO (mL/min)=1 (cpm)/SR (mL/min/RS(cp)m) and CBF (mL/mm)=LV (cpm)/SR (mL/min)/RS (cpm), where I is total radioactivity injected and was calculated as the difference in counts in the tubing before and after injection of the microspheres, RS is counts in the reference blood sample, SR is rate of withdrawal from the reference arteries, and 1 is LV count. Cardiac index and TPRI were calculated and expressed as mL/min/kg and U, respectively. CVR was calculated by dividing mean arterial pressure (MAP), recorded just before each microsphere injection, by its corresponding CBF. The CBF and CVR were normalized with respect to RV and LV weight (CBF per gram of myocardium and CVR) and expressed as mL/mm/g and U, respectively. A total of 15 rats were discarded from the study because of unsuccessful cannulation, uneven microsphere distribution to both kidneys, or pulmonary AV shunting.

Protocol
After full recovery from anesthesia, the rats were studied while unrestrained in a open-top plastic cage. Both the caudal arterial and LV catheters were connected to Gould-Statham pressure transducers (1273D by Statham Instruments) MAP and HR were recorded throughout the study on a multichannel physograph coupled with a cardiotachometer (Sensor Medics R612). Baseline (control) measurements were obtained after 30 minutes of hemodynamic stabilization using a first microsphere (\(^{113}\)Co) injection. After these baseline measurements were obtained, each rat ran on the treadmill starting at a speed of 0.4 m/s that was increased at a rate of 0.15 m/s every minute until exhaustion, indicated by inability to maintain a pace with the treadmill. The second microsphere (\(^{113}\)Sn) injection was given at the peak of maximal exercise. Then, after full recovery from exercise (60 minutes), maximal coronary vasodilatation was produced by dipyridamole, administered (4 mg kg\(^{-1}\) min\(^{-1}\) IV for 10 minutes) using a Harvard infusion/withdrawal pump (Harvard Apparatus). Maximal CBF was then determined by injecting the third radiomolecule microsphere (\(^{46}\)Sc) while the dipyridamole was infused continually for 1 to 2 minutes after the microspheres were injected.

Statistical Analysis
All results are expressed as mean±SEM. The paired Student's t-test was used for group comparisons (baseline versus treadmill or baseline versus dipyridamole). Single-factor ANOVA was carried out to assess differences between treatment groups. The mean change from baseline (with treadmill or dipyridamole) was expressed as a CVR and was tested for statistical significance using ANCOVA with treatment group as a factor and the baseline as a covariate. When the F statistic from ANOVA or ANCOVA was significant, pairwise comparisons were made using the Student-Newman-Keuls post-hoc test to adjust for multiplicity of testing. Correlation coefficients were computed using a least-squares linear regression analysis. Data analyses and calculations were accomplished using JMP version 3.1.5 statistical software (SAS Institute Inc). The level of significance was defined at an α=0.05.

Results

Body, Aortic, and Cardiac Mass
Body weight was significantly less in untreated SHR than normotensive WKY-C (P<0.05), however, there were no differences among the treated SHR groups with respect to the untreated SHR (Table 1). There were no differences in RV mass among the WKY and SHR untreated groups, however, it was significantly reduced in the three treated groups (Table 1). Aortic mass index was significantly lower in the WKY-C than the SHR-C, and all treatments diminished aortic mass index in SHR groups (without differences between treatment groups). LV mass was markedly increased in untreated SHR rats (compared with WKY (P<0.01) and all three SHR treatment groups (P<0.01, each group). The combination therapy reduced LV mass (by 21%) more than enalapril (17%, P<0.05) or losartan (15%, P<0.01) alone LV mass and MAP of the SHR control and treatment groups were directly correlated (r=0.670, P<0.01).

Systemic Hemodynamics
MAP and TPRI were significantly increased in the SHR-C (148±5 versus 98±6 mm Hg, P<0.01, and 0.385±0.032 versus 0.240±0.015 U, P<0.01, respectively) with respect to the WKY-C. Moreover, MAP was reduced by enalapril (20%, P<0.01), losartan (20%, P<0.01), and their combination (34%, P<0.01); the latter was reduced more than either drug alone (P<0.05). This MAP reduction was achieved through a significant decrease in TPRI, there were no differences between groups in heart rate or cardiac index (Table 2).

Coronary Hemodynamics
Resting CBF (per unit of RV and LV mass) did not differ among the various SHR groups. Because MAP was lower in WKY-C and was significantly reduced by treatment in the SHR groups, CVRI was also reduced in these
groups. However, only the untreated WKY and the combination treatment were significantly lower than the SHR-C (both P<0.01). The distribution of CBF in subendocardial and subepicardial layers (expressed as a ratio) showed no differences among the groups (Table 2).

**Exercise and Dipyridamole Interventions**

MAP was reduced in all groups except SHR-C by dipyridamole (P<0.01) and with treadmill exercise (Table 3). Treadmill exercise induced a moderate but significant fall in TPR that was associated with a slightly faster heart rate in all treated groups; however, only the group receiving both enalapril and losartan achieved significant increases in both heart rate and cardiac index. Dipyridamole infusion increased LV CBF in all groups (P<0.01) except the SHR-C CBF reserve (defined as the difference between maximal CBF achieved with dipyridamole and the baseline CBF) was significantly greater with SHR-L and SHR-E&L (323±0.68 mL min⁻¹ g⁻¹, P<0.05, and 3.80±0.45 mL min⁻¹ g⁻¹, P<0.01, respectively) than in the SHR-C (1.47±0.39 mL min⁻¹ g⁻¹). CVRI was reduced in all groups by dipyridamole (P<0.01); however, the CVR (expressed as the ratio between MCVR and CVRI at baseline) was significantly reduced by SHR-L and SHR-E&L (0.55±0.06, P<0.05, and 0.52±0.04, P<0.01, respectively) in comparison with the SHR-C (0.67±0.05). Minimal CVR, achieved after maximal dilation with dipyridamole, was reduced in all three treatment groups as well as with the WKY-C (P<0.01 for each). During treadmill exercise, there was a similar trend in coronary hemodynamics, although exercise was far less effective than pharmacological intervention (Table 4). Finally, LV CFR with dipyridamole was inversely related to basal MAP and LV mass (r=-0.40, P<0.01, and r=-0.43, P<0.01, respectively).

**Discussion**

The results of this study demonstrated that long-term AT₁ receptor blockade alone or in combination with ACE inhibition was effective in improving coronary hemodynamics associated with reduction in cardiac mass related to LVH. Three lines of evidence support this conclusion: (1) enalapril and losartan equally reduced MAP and LV mass; (2) the combination of enalapril and losartan (in half-doses of each when used alone) reduced pressure and LV mass more than either drug administered alone, and (3) although enalapril had no effect on coronary hemodynamics, only losartan or the combination was more effective in normalizing coronary hemodynamics (to values closer to that of normotensive WKY).

ACE inhibitors as well as AT₁ receptor antagonists are extremely effective in reducing arterial pressure; however, it is not clear why the simultaneous use of both agents was more effective than either agent when used alone in twice the dose of each. Furthermore, this greater effect of the combined therapy was reduced LV mass more than either drug when used alone. Prior studies from our and other laboratories using ACE inhibitors demonstrated reduction of cardiac mass even when arterial pressure was suboptimally reduced, suggesting nonhemodynamic effects of...
Table 3. Systemic Hemodynamic Indices Obtained After Treadmill Exercise or Dipyridamole Infusion

<table>
<thead>
<tr>
<th></th>
<th>WKY-C (n=10)</th>
<th>WKY-C (n=12)</th>
<th>SHR-E (n=11)</th>
<th>SHR-L (n=13)</th>
<th>SHR-E&amp;L (n=16)</th>
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<tbody>
<tr>
<td>Time</td>
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<td></td>
<td>B</td>
<td>T</td>
<td>D</td>
<td>B</td>
<td>T</td>
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<td>Heart rate, bpm</td>
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<tr>
<td>B</td>
<td>395±16*</td>
<td>413±14§</td>
<td>375±15*</td>
<td>415±19</td>
<td>468±33</td>
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<tr>
<td>T</td>
<td>413±14§</td>
<td>448±13</td>
<td>431±14</td>
<td>409±33</td>
<td>436±30</td>
</tr>
<tr>
<td>D</td>
<td>73±5*‡</td>
<td>123±6‡</td>
<td>96±5*‡</td>
<td>419±35</td>
<td>482±41</td>
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<td>Cardiac index, mL min⁻¹ kg⁻¹</td>
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<tr>
<td>B</td>
<td>53±28</td>
<td>27±45</td>
<td>53±42</td>
<td>53±28</td>
<td>53±42</td>
</tr>
<tr>
<td>T</td>
<td>468±33</td>
<td>436±30</td>
<td>509±26</td>
<td>482±41</td>
<td>514±25§</td>
</tr>
<tr>
<td>D</td>
<td>0 240±0 015*</td>
<td>0 385±0 029</td>
<td>0 300±0 026†</td>
<td>0 282±0 026*</td>
<td>0 248±0 016*</td>
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<td>TPRU, U</td>
<td></td>
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<tr>
<td>B</td>
<td>0 200±0 015*</td>
<td>0 385±0 029</td>
<td>0 300±0 026†</td>
<td>0 282±0 026*</td>
<td>0 248±0 016*</td>
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<tr>
<td>T</td>
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<td>0 321±0 021</td>
<td>0 230±0 027*</td>
<td>0 213±0 015§</td>
<td>0 181±0 014‡</td>
</tr>
<tr>
<td>D</td>
<td>0 155±0 016‡</td>
<td>0 346±0 025</td>
<td>0 215±0 020‡</td>
<td>0 207±0 017§</td>
<td>0 183±0 013‡</td>
</tr>
<tr>
<td>B-T</td>
<td>0 029±0 011</td>
<td>0 060±0 039</td>
<td>0 070±0 025</td>
<td>0 066±0 021</td>
<td>0 067±0 016</td>
</tr>
</tbody>
</table>

Values are mean±SEM. B indicates baseline, T, during treadmill exercise, and D, during dipyridamole infusion.

Table 4. LV Coronary Hemodynamic Indexes Obtained After Treadmill Exercise or Dipyridamole Infusion

<table>
<thead>
<tr>
<th></th>
<th>WKY-C (n=10)</th>
<th>WKY-C (n=12)</th>
<th>SHR-E (n=11)</th>
<th>SHR-L (n=13)</th>
<th>SHR-E&amp;L (n=16)</th>
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<tr>
<td>Time</td>
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<td></td>
<td>B</td>
<td>T</td>
<td>D</td>
<td>B</td>
<td>T</td>
</tr>
<tr>
<td>CBF, mL min⁻¹ g⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>6 16±0 26</td>
<td>5 86±0 34</td>
<td>5 53±0 28</td>
<td>5 50±0 46</td>
<td>5 63±0 29</td>
</tr>
<tr>
<td>T</td>
<td>6 92±0 53*</td>
<td>6 64±0 57</td>
<td>6 69±0 44</td>
<td>7 52±0 68$</td>
<td>7 20±0 73*</td>
</tr>
<tr>
<td>D</td>
<td>10 76±0 43*</td>
<td>7 12±0 41</td>
<td>7 63±0 42§</td>
<td>6 74±0 60$</td>
<td>9 46±0 56*</td>
</tr>
<tr>
<td>CRF, mL min⁻¹ g⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-T</td>
<td>3 10±0 87*</td>
<td>1 01±0 86</td>
<td>0 36±0 54</td>
<td>2 01±0 64</td>
<td>1 55±0 88</td>
</tr>
<tr>
<td>B-D</td>
<td>4 55±0 59*</td>
<td>1 47±0 39</td>
<td>2 26±0 63$</td>
<td>3 23±0 68$</td>
<td>3 80±0 45</td>
</tr>
<tr>
<td>CFVU, U</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>16 1±1 56</td>
<td>27 1±1 58</td>
<td>23 9±1 68</td>
<td>23 4±1 12</td>
<td>17 8±0 70*</td>
</tr>
<tr>
<td>T</td>
<td>10 9±1 01*</td>
<td>21 6±1 70$</td>
<td>18 9±1 69</td>
<td>15 1±1 14$</td>
<td>14 0±1 44*</td>
</tr>
<tr>
<td>D</td>
<td>6 0±0 60*</td>
<td>17 7±1 21</td>
<td>10 0±1 91*</td>
<td>12 0±0 84$</td>
<td>9 0±0 64*</td>
</tr>
<tr>
<td>CVR reserve</td>
<td>T/B</td>
<td>0 66±0 07</td>
<td>0 84±0 08</td>
<td>0 87±0 09</td>
<td>0 79±0 08</td>
</tr>
<tr>
<td></td>
<td>D/B</td>
<td>0 43±0 03*</td>
<td>0 67±0 06</td>
<td>0 58±0 05</td>
<td>0 55±0 06†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Abbreviations as in Table 3.

Inhibition of the generation of angiotensin II by losartan may also be an operating mechanism. As previously stated, hypertensive LVH is associated with impaired coronary hemodynamics, manifested by reduced CRF, reduced CFR, elevated MCVR, and abnormal subendocardial autoregulation. Reduced CFR in LVH has been attributed to structural alterations of the coronary resistance vessels (vascular component) manifested by a reduction in the force of arterial vasculature, capillary density, and decreased total cross-sectional area of resistance vessels. The myocardial component in LVH may also contribute to an extravascular component of the increased CVR that is manifested by forces related to myocardial contractility and relaxation and by interstitial and perivascular myocardial fibrosis. Although enhanced activity of endothelium-derived vasoconstricting factor may also be an operating mechanism, recent studies have demonstrated impaired endothelial-dependent coronary vasodilatation in response to acetylcholine in hypertensive patients, although enhanced activity of endothelium-derived vasoconstricting factor may also be an operating mechanism. In addition, attenuation of endothelial-mediated control of CVR may also exist. Recent studies have demonstrated impaired endothelial-dependent coronary vasodilatation in response to acetylcholine in hypertensive patients, although enhanced activity of endothelium-derived vasoconstricting factor may also be an operating mechanism. In addition, attenuation of endothelial-mediated control of CVR may also exist. Recent studies have demonstrated impaired endothelial-dependent coronary vasodilatation in response to acetylcholine in hypertensive patients, although enhanced activity of endothelium-derived vasoconstricting factor may also be an operating mechanism. In addition, attenuation of endothelial-mediated control of CVR may also exist.
only 3 weeks did not demonstrate a significant improvement in overall coronary hemodynamics, although the reductions in MAP (23%) and LV mass (19%) were comparable to the present study. This, the results of the present study indicate that more than 3 weeks of treatment was necessary to improve all coronary hemodynamic parameters.\(^{22}\)

Thus, the present findings also support the hypothesis that Angiotensin II, through its effects on AT\(_1\) receptors, may participate importantly in reducing the CBF reserve associated with LVH. However, by adding enalapril to the losartan (even in half doses of each), the coronary hemodynamic effects were improved further and more significantly. This could be explained by the dual effects of inhibiting the renin-angiotensin system and the additional effect of ACE inhibition on increasing the role of ki

**Acknowledgments**

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