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

Eduardo Nunez, Kazuyoshi Hosoya, Dunko Susc, 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 diltiation (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 impaired 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 synergism • losartan • enalapril • coronary circulation • spontaneously hypertensive rats
blood flows to the right and left kidneys; a difference of \( \pm 15\% \) 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 animal was 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, subepicardial and subendocardial, to assess regional myocardial perfusion. These segments were carefully cleaned, blotted dry, and weighed on a precision balance. Ventricular weights were normalized for body weight and expressed as aortical 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 internal inversion software (CompuSphere, Packard). Thus, CO and CBF were calculated using these formulas: 

\[
CO = (mL/min/1000) \times SR (cpm/mL/mm) \\
CBF = (mL/min) \times LV (cpm) \times SR (mL/mm) \\
\]

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 LV 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) by CBF, recorded just before each microsphere injection, by its corresponding CBF. CO and CBF were calculated using the formula: 

\[
CO = (MHR \times DBP \times SV) / 100 \\
CBF = (MHR \times SBP \times SV) / 100 \\
\]

where MHR is mean heart rate and DBP and SBP are diastolic and systolic blood pressure, respectively.

**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 (123D by Statham Instruments) MAP and HR were recorded throughout the study on a multichannel physiograph coupled with a cardiotachometer (Sensor Medics R612). Baseline (control) measurements were obtained after 30 minutes of hemo-dynamic stabilization using a first microsphere (\(^{14}Co\) 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 (\(^{114}Sn\) 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\(^{-1}\) \(\times\) 10 minutes) using a Harvard infusion/withdrawal pump (Harvard Apparatus). Maximal CBF then was determined by injecting the third radionuclide 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) is 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 \(F\) 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 analysis and calculations were accomplished with JMP version 3.1.5 statistical software (SAS Institute Inc). The level of significance was defined at an \( \alpha = 0.05 \).

**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 \((r = 0.670, P<0.05)\).

**Systemic Hemodynamics**

MAP and TPRI were significantly increased in the SHR-C \((148±5 vs 98±6 \text{ mm Hg, } P<0.01, 0.385±0.032 versus 0.240±0.015 U, P<0.01, respectively)\) 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
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 TPRI 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 basal CBF) was significantly greater with SHR-L and SHR-E&L (3.23±0.68 mL/min·g⁻¹, P<0.05, and 3.80±0.45 mL/min·g⁻¹, P<0.01, respectively) than in the SHR-C (1.47±0.39 mL/min·g⁻¹, P<0.01). CVRI was reduced in all groups by dipyridamole (P<0.01 for each); 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 a value 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...
inhibition of the generation of angiotensin II \textsuperscript{[10,21,25-27]} or an additional effect of kinase associated with ACE inhibition.\textsuperscript{[8,32]} However, the latter mechanism seems less likely to be operative in the present study since losartan was at least equally as effective or more effective than enalapril when either was used alone. Supporting the primary hemodynamic effect of losartan in reducing LV mass (as opposed to a nonhemodynamic action) are the findings that AT\textsubscript{1} receptors in contrast to the AT\textsubscript{2} receptors mediate the mitogenic effects of angiotensin II \textsuperscript{33,34} And further support of this line of reasoning is the highly positive correlation that we demonstrated between LV mass and MAP (r=0.67, P<0.01), although nonhemodynamic factors may still participate. Thus, enalapril and losartan equally reduced RV as well as LV mass, although the combination treatment was even more effective (Table 1). This reduction in overall cardiac mass may also be related to a decrease in LV and RV wall collagen content, and while this was not determined in this study, we did show in earlier work that ACE inhibition did in fact diminish RV as well as LV collagen \textsuperscript{35,36}.

The present study also demonstrated that associated with the reduction in MAP and LV mass, losartan improved coronary hemodynamics, although the combination with enalapril was even more effective. As already stated, hypertensive LVH is associated with impaired coronary hemodynamics, manifested by reduced CRF, reduced CFR, elevated MCVR, and abnormal subendocardial autoregulation.\textsuperscript{5-9} Reduced CFR in LVH has been attributed to structural alterations of the coronary resistance vessels (vascular component) manifested by a reduction in reduced arterial vasculanty, capillary density, and decreased total cross-sectional area of resistance vessels \textsuperscript{37-41}. The myocardial component in LVH may also contribute to an extravascular component of the increased CVR that is manifested by forces related to myocardial contraction and relaxation and by interstitial and perivascular myocardial fibrosis \textsuperscript{41,42}. In addition, attenuation of endothelially mediated control of CVR may also exist. Recent studies have demonstrated impaired endothelially dependent coronary vasodilatation in response to acetylcholine in hypertensive patients,\textsuperscript{43-45} although enhanced activity of endothelium-derived vasocostraining factor may also be an operating mechanism.\textsuperscript{46} Additionally, several reports have suggested that abnormalities in coronary circulation persist even after LVH reversal and MAP reduction, suggesting a dissociation between LV mass and coronary vessel abnormalities in response to antihypertensive treatment \textsuperscript{21,47,48}. In support of this thesis is a recent study from our laboratory that was conducted with the same protocol used in the present study. In that study, we demonstrated that losartan administered for

### Table 3. Systemic Hemodynamic Indices Obtained After Treadmill Exercise or Dipyridamole Infusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WKY (n=10)</th>
<th>WKY (n=12)</th>
<th>SHR-E (n=11)</th>
<th>SHR-L (n=13)</th>
<th>SHR-E&amp;L (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>96±5*</td>
<td>134±3</td>
<td>101±3§</td>
<td>105±5*§</td>
<td>89±4*</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>395±16</td>
<td>426±9</td>
<td>421±11</td>
<td>440±9</td>
<td>400±10</td>
</tr>
<tr>
<td>Cardiac index, mL min\textsuperscript{-1} kg\textsuperscript{-1}</td>
<td>416±14§</td>
<td>448±13</td>
<td>466±14§</td>
<td>469±11§</td>
<td>434±8§</td>
</tr>
<tr>
<td>Time</td>
<td>WKY-C (n=10)</td>
<td>WKY-C (n=12)</td>
<td>SHR-E (n=11)</td>
<td>SHR-L (n=13)</td>
<td>SHR-E&amp;L (n=16)</td>
</tr>
<tr>
<td>D</td>
<td>53±28</td>
<td>27±45</td>
<td>53±42</td>
<td>57±32</td>
<td>104±33</td>
</tr>
<tr>
<td>B-T</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>
</tr>
<tr>
<td>B</td>
<td>0.210±0.019*</td>
<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>0.083±0.039</td>
<td>0.070±0.025</td>
<td>0.068±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>Parameter</th>
<th>WKY (n=10)</th>
<th>WKY (n=12)</th>
<th>SHR-E (n=11)</th>
<th>SHR-L (n=13)</th>
<th>SHR-E&amp;L (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF, mL min\textsuperscript{-1} g\textsuperscript{-1}</td>
<td>6.15±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>CRF, mL min\textsuperscript{-1} g\textsuperscript{-1}</td>
<td>9.26±0.53†</td>
<td>6.84±0.57</td>
<td>5.68±0.44</td>
<td>7.52±0.68†</td>
<td>7.20±0.73‡</td>
</tr>
<tr>
<td>CRFVI, U</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±0.36*</td>
</tr>
<tr>
<td>CBF 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>0.66±0.07</td>
</tr>
<tr>
<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>
<td>0.52±0.04*</td>
</tr>
</tbody>
</table>

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

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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. Thus, the results of the present study indicate that more than 3 weeks of treatment was necessary to improve all coronary hemodynamics and indexes (Table 4), a finding that may explain negative results in short-term trials. On the other hand, we deliberately used a 3-week treatment period in our earlier studies to dissociate hemodynamic from nonhemodynamic effects produced by antihypertensive therapy. Thus, the present findings also support the hypothesis that angiotensin II, through its effects on AT, 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 kinins. This concept is supported by the finding that losartan, after 12 weeks of treatment, normalized MAP, reduced LV mass, and improved coronary hemodynamics, although not to the extent achieved in combination with enalapril. Finally, it is important to recognize that despite the controlled MAP, reduction in LV mass, and improved coronary hemodynamics associated with AT1 receptor antagonism, reduction in cardiac risk was not a feature of the present experimental design.

Acknowledgments

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