Effects of Losartan + L-Arginine on Nitric Oxide Production, Endothelial Cell Function, and Hemodynamic Variables in Patients With Heart Failure Secondary to Coronary Heart Disease

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The purpose of the present study was to evaluate the effects of losartan and the combination of losartan and L-arginine on endothelial function and hemodynamic variables in patients with heart failure (HF). Endothelium-dependent vasodilation is impaired in patients with HF. It was hypothesized that the administration of losartan and the combination of losartan and L-arginine might increase nitric oxide production and have a beneficial additive effect on endothelial function and hemodynamic variables in patients with HF. Nine patients with HF (ejection fraction <35%) were given losartan 50 mg orally on 2 consecutive days. On the second day, 1 hour after losartan 50 mg administration, L-arginine 20 g was given by intravenous infusion. Endothelial function in the form of endothelium-dependent brachial artery flow-mediated vasodilation (FMV) was measured by ultrasound. Hemodynamic variables were estimated using Doppler echocardiography at baseline and at 2 and 4 hours after losartan alone and after combination therapy. Urinary levels of nitrite (NO2) or nitrate (NO3) were measured. Four hours after losartan administration, significant reductions in systemic vascular resistance and estimated end-systolic elastase were observed. On the second day, 1 hour after L-arginine infusion, an additive hemodynamic effect was observed, with significant increases in the cardiac index and stroke volume and significant reductions in systemic vascular resistance and calculated left ventricular end-diastolic pressure. A trend toward improved FMV was observed with losartan alone, but without statistical significance. Combination therapy significantly improved postintervention FMV compared with baseline. The increase in urinary nitric oxide excretion after losartan treatment and combination therapy was significantly correlated with improved hemodynamic variables and improved FMV. In conclusion, losartan induces significant afterload reduction, reduced contractility, and increased nitric oxide urinary excretion. The combination of L-arginine and losartan seems to have superior effects on hemodynamic variables and endothelium-dependent vasodilation compared with losartan alone. © 2006 Elsevier Inc. All rights reserved. (Am J Cardiol 2006;98:172–177)

This study evaluated the immediate hemodynamic response and effect on endothelial function of the use of losartan, an angiotensin-1 receptor antagonist, and of the combination of losartan and L-arginine in patients with heart failure (HF) using noninvasive echocardiographic studies and correlated these responses with changes in urinary nitrite (NO2) or nitrate (NO3), which are stable metabolites of nitric oxide.1,2 Furthermore, because angiotensin-1 receptor antagonists supposedly increase nitric oxide bioavailability by augmenting epithelial nitric oxide synthase expression and activity,3–5 we hypothesized that a combination of an angiotensin-1 receptor antagonist and intravenous L-arginine might be additive with regard to correcting endothelial dysfunction in patients with HF.

Methods

The study group comprised 9 patients with HF in stable condition whose symptoms were compatible with New York Heart Association functional classes II or III. All patients had HF due to coronary heart disease, and ejection fractions by radionuclide angiography ranged from 19% to 30%. After providing written informed consent, the patients were admitted to the coronary care unit 1 day before the study. All patients were in sinus rhythm. We excluded patients with active anginal syndrome, recent myocardial infarctions, coronary artery bypass surgery, or any systemic chronic diseases. All patients were treated with digitalis and furosemide, which were withheld on the morning of the study. Six patients were receiving angiotensin-converting enzyme inhibitors, and 3 patients were treated with angiotensin II type 1 receptor antagonists. Vasodilatory therapy with an angiotensin-converting enzyme inhibitor, an angio-
tensin II inhibitor, or nitrates was withdrawn 48 hours before the study.

Noninvasive hemodynamic studies: A Siemens C256 imaging system (Acuson, Mountain View, California) was used for imaging and Doppler flow studies. For volume estimation, the apical 4-chamber view was used, as previously described.6–9 Maximal effort was made to ensure the maximal length and width of the left ventricle. Images were accepted for analysis if ≥75% of the endocardium was seen. All measurements were performed at baseline before drug administration and at 2 and 4 hours after losartan administration. A separate examination was performed on the second day of the study within 60 and 180 minutes of l-arginine administration (2 and 4 hours after losartan administration on the second day). Tracing was performed with a built-in computer for single-plane area, length, and volume calculations and Simpson’s rule computation from tracings of the ventricular outlooks. Ventricular volumes were measured at end-diastole and at end-systole. Left ventricular end-systolic pressure was estimated by the formula: left ventricular end-systolic pressure = peripheral systolic pressure – 10 mm Hg, as previously described.10 The left ventricular ejection fraction was calculated from end-diastolic volume and end-systolic volume by the standard formula: left ventricular ejection fraction = (left ventricular end-diastolic volume – left ventricular end-systolic volume)/left ventricular end-diastolic volume. Cardiac output was calculated by forward aortic flow using pulsed Doppler echocardiography. For the recording of left ventricular outflow at the level of the aortic annulus, the transducer was placed at the apical area and rotated toward the long-axis or hemi-axial view. The sample volume was placed in the middle of the left ventricular outflow tract immediately proximal to the leaflet of the aortic valve, as previously described.11 Forward stroke volume was determined as the product of the time–velocity integral (average diastolic pressure (LVEDP) and left ventricular end-diastolic pressure estimated from jugular venous pressure. The formula used was SVR = (mean blood pressure – jugular venous pressure) × 80/cardiatic output (dyne's s⁻¹ cm⁻⁵)

In this trial, we used elastase end-systolic (Ees) approximation as a measure of contractility. Many single-beat estimation methods to measure Ees as a measure of contractility have been reported. The simplest and most frequently used method18,19 is to ignore V₀ and report the ratio of end-systolic pressure to end-systolic volume. All Doppler and echocardiographic analyses were performed by 2 operators who did not know at which stage of the study the recordings were obtained. All data presented are the averages of the measurements obtained by the 2 operators.

Endothelial function in the form of endothelium-dependent brachial artery flow-mediated vasodilatation (FMV) was measured as previously described.20–22 Ultrasound measurements of the brachial artery were performed in the supine condition, at the elbow of the right arm, using a vessel wall movement system consisting of an ultrasound imager with a 10-MHz linear-array transducer connected to a data acquisition system and a personal computer (Hewlett-Packard Corporation, Palo Alto, California). By the inflation of a blood pressure cuff for 4 minutes at a pressure of 40 mm Hg greater than systolic blood pressure, ischemia was applied to the forearm distal to the location of the transducer. Brachial artery diameter was measured by Doppler ultrasonography at baseline and 60 and 90 seconds after the release of the forearm blood pressure cuff. The diameter changes caused by endothelium-dependent FMV (percentage FMV) are expressed as the percentage changes relative to those at the initial scan at rest.

On the first day, losartan was given orally at a dose of 50 mg, as previously described in the Evaluation of Losartan in the Elderly trial.23 On the next day, 1 hour after losartan 50 mg administration, we started a continuous 1-hour infusion of 100 ml of 5% glucose mixed with 100 ml of 20% solution of L-arginine hydrochloride through the antecubital vein, as previously described; a total of 20 g of L-arginine was administered at a constant rate. Heart rate and blood pressure were recorded every hour during the study and every 15 minutes during the L-arginine infusion. Blood and urine samples were obtained at baseline and at 1, 2, and 4 hours after losartan administration on the 2 days. In all urinary and blood samples, NO₂ or NO₃, creatinine, and sodium levels were determined as previously described.7 All Doppler echocardiographic and peripheral vascular studies were repeated at baseline (at the entry of the patients into the study, before losartan administration) and at 2 and 4 hours after losartan administration on the 2 days.

Statistical analysis: Data are expressed as mean ± SD. Paired data were compared using Student’s t test. Pearson’s
correlation coefficient was used to assess the association of clinical and echocardiographic variables with symptomatic response. A p value <0.05 was considered significant.

Results

Baseline characteristics: The baseline characteristics of the study group are listed in Table 1.

Effect of losartan on hemodynamic indexes: The changes in hemodynamic variables after losartan are depicted in Figure 1. Losartan administration resulted in significant reductions in mean blood pressure (p = 0.02), SVR (p = 0.04), and Ees (p = 0.02). Although trends toward increased cardiac index, increased stroke volume, and reduced calculated LVEDP were observed, these changes did not reach statistical significance. There were no significant differences in diastolic indexes after losartan treatment.

Effects of combination therapy of L-arginine and losartan on hemodynamic indexes: The changes in hemodynamic variables after combination therapy (1 hour after the end of L-arginine infusion and 2 hours after combination therapy) are depicted in Figure 1. In comparison with losartan alone, combination therapy improved hemodynamic variables significantly. SVR decreased from 2,365 ± 889 dynes · s⁻¹ · cm⁻⁵ 2 hours after losartan alone to 1,900 ± 560 dynes · s⁻¹ · cm⁻⁵, 2 hours after combination therapy (p = 0.005; Figure 1), and from 2,194 ± 749 dynes · s⁻¹ · cm⁻⁵ 4 hours after losartan alone to 1,840 ± 605 dynes · s⁻¹ · cm⁻⁵ 4 hours after combination therapy (p = 0.04). Estimated Ees further decreased from 0.69 ± 0.21 mm Hg/ml 2 hours after losartan alone to 0.54 ± 0.17 mm Hg/ml 2 hours after combination therapy (p = 0.007; Figure 1), implying reduced contractility with combination therapy. The cardiac index improved from 1.8 ± 0.47 L/min/m² 2 hours after losartan alone to 2.03 ± 0.51 L/min/m² 2 hours after combination therapy (p = 0.0007; Figure 1) and from 1.86 ± 0.44 L/min/m² 4 hours after losartan alone to 2.18 ± 0.57 L/min/m² 4 hours after combination therapy (p = 0.04). Stroke volume increased from 59.5 ml 2 hours after losartan alone to 65.5 ± 17.4 ml 2 hours after combination therapy (p = 0.001; Figure 1). Calculated LVEDP was reduced from 21.2 ± 1.8 mm Hg 2 hours after losartan alone to 6.9 ± 2.1 mm Hg after combination therapy (p <0.0001; Figure 1).

Effects of losartan therapy and combination therapy on endothelial dysfunction: The effects of losartan and combination therapy on FMV are shown in Figure 2. Although a trend toward improved FMV was observed with losartan treatment alone, these changes did not reach statistical significance. At baseline, the study group had FMV of 14.3 ± 7.7%. Four hours after losartan treatment alone, FMV had improved (22.5 ± 11%), but the difference did not reach statistical significance (p = 0.06). Four hours after combination therapy, FMV had improved significantly to 27.8 ± 11.3% (p = 0.01) compared with baseline measurements, but the comparison between FMV 2 and 4 hours after losartan alone and FMV 2 and 4 hours after combination therapy did not reach statistical significance.

Nitrite or nitrate changes: The changes in urinary nitric oxide excretion 4 hours after losartan treatment (day 1) are shown in Figure 3. NO₂ or NO₃ urinary excretion increased significantly to 0.9 ± 0.6 nmol/mg creatinine from the baseline measurement of 0.6 ± 0.39 nmol/mg creatinine (p = 0.03). The increased NO₂ or NO₃ urinary excretion 4 hours after losartan administration was positively and significantly correlated with stroke volume (R = 0.65, p <0.005) and negatively and significantly correlated with SVR (R = −0.42, p <0.05) and Ees (R = −0.44, p <0.05). The changes in urinary nitric oxide excretion after combination therapy (day 2) and 60 and 180 minutes after the end of L-arginine administration are depicted in Figure 3. NO₂ or NO₃ urinary excretion increased significantly to 1.05 ± 0.44 nmol/mg creatinine from the pretreatment value of 0.58 ± 0.3 nmol/mg creatinine (p = 0.0002) 60 minutes after the end of L-arginine administration. The increased NO₂ or NO₃ urinary excretion 60 minutes after L-arginine administration was positively and significantly correlated with stroke volume (R = 0.56, p <0.01) and negatively and significantly correlated with SVR (R = −0.46, p <0.05) and Ees (R = −0.49, p <0.05).

Table 1

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yrs)/Sex</th>
<th>EF (%)</th>
<th>CI (L/min/m²)</th>
<th>SVR (dynes · s⁻¹ · cm⁻⁵)</th>
<th>LVEDV (ml)</th>
<th>Estimated Ees (mm Hg/ml)</th>
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<tr>
<td>1</td>
<td>61F</td>
<td>28</td>
<td>2.2</td>
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<td>2</td>
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<tr>
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<tr>
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<tr>
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<td>153</td>
<td>0.86</td>
</tr>
<tr>
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<td>0.95</td>
</tr>
<tr>
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<td>1.29</td>
<td>4.284</td>
<td>200</td>
<td>0.7</td>
</tr>
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<td>Average</td>
<td>69 ± 5.1</td>
<td>26.8 ± 3.7</td>
<td>1.7 ± 0.4</td>
<td>2,444 ± 876</td>
<td>197 ± 57</td>
<td>0.71 ± 0.19</td>
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CI = cardiac index; EF = ejection fraction; LVEDV = left ventricular end-diastolic volume.
The changes in the mean value of NO₂ or NO₃ excretion 3 hours after the end of L-arginine administration remained greater than the preinfusion value (0.98 ± 0.5 nmol/mg creatinine, p = 0.005).

**Discussion**

This study demonstrates that angiotensin-1 receptor antagonists reduce afterload variables (mean blood pressure and SVR). Although reduced SVR is probably mainly mediated through antagonism of the potent vasoconstrictor effect of angiotensin through its type 1 receptor on peripheral vascular vessels, the significant correlations found between increased NO₂ or NO₃ urinary excretion (nanomoles per milligram of creatinine) and reduced SVR may suggest a secondary effect of the drug through increased nitric oxide production. The calculation of Ees in our study suggests reduced contractility after treatment with losartan. Interestingly, a similar effect was found by Wittstein et al with angiotensin-converting enzyme inhibitors. The proposed mechanism for reduced contractility with angiotensin-converting enzyme inhibitors was increased myocardial nitric oxide production. Nitric oxide attenuates the positive inotropic response to β-adrenergic stimulation in humans and in animal models, and this effect appears to be augmented in the setting of left ventricular dysfunction. In this study, we found a similar effect of angiotensin-1 receptor antagonists. Although we did not use epithelial nitric oxide synthase inhibitors, the negative significant correlation be-

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**Figure 1.** (A to F) Changes in the hemodynamic variables after losartan and after combination therapy. Losartan administration resulted in a significant reduction in afterload indexes (blood pressure (A) and SVR (B)): interestingly, combination therapy further reduced SVR. (C) Estimated Ees decreased significantly after losartan administration, implying reduced contractility. Combination therapy further reduced estimated Ees. (D to F) Hemodynamic changes after combination therapy. Combination therapy led to improved variables of integrated cardiac performance (cardiac index [CI] (D) and stroke volume [SV] (E)). (F) Combination therapy led to enhanced left ventricular chamber compliance, as implied by reduced calculated LVEDP.
between NO$_2$ or NO$_3$ urinary excretion and estimated Ees suggests a role for nitric oxide in the negative inotropic effect of angiotensin-1 antagonism. These previously unappreciated properties of angiotensin-1 receptor antagonists may contribute to their therapeutic effects in patients with HF by reducing the heart’s oxygen consumption and protecting the heart from toxic influence of β stimulation.

**Effects of a combination of losartan and intravenous L-arginine supplementation:** This part of the study demonstrates that the combination of oral losartan and intravenous L-arginine has additive effects compared with losartan alone. Combination therapy leads to improved variables of integrated cardiac performance—cardiac index and stroke volume as well as reduction in afterload indexes—more than losartan alone. This occurred concomitantly with improved endothelial function and significant improvement in diastolic properties. Combination therapy reduced contractility (estimated Ees) even more than losartan alone. Stroke volume was positively and significantly correlated with NO$_2$ or NO$_3$ urinary excretion, and SVR and Ees were negatively and significantly correlated with NO$_2$ or NO$_3$ urinary excretion. These correlations imply a possible role for nitric oxide production in the mechanism of the hemodynamic effects of combination therapy in patients with HF. Interestingly, the results 4 hours after combination therapy compared with 4 hours after losartan alone were less impressive than the results comparing the regimens 2 hours after treatment. Although the differences in SVR and cardiac index retained statistical significance, only trends toward decreased Ees, increased stroke volume, and reduced calculated LVEDP were observed 4 hours after combination therapy, not reaching statistical significance. The reduced potency of combination therapy 3 hours after the end of L-arginine infusion probably reflects the short-lived effect of L-arginine given intravenously.

Several limitations warrant mention. The methods of measurements were noninvasive, the hemodynamic changes were relatively small, and the effects of losartan and L-arginine were not evaluated in a dose-response manner. A control group comprising patients treated with L-arginine alone was not part of the study design. The measure of contractility was estimated Ees, based on the assumption that $V_0$ is minimal. This method is problematic because there are marked differences in $V_0$, with often large nonzero intercepts, in patients with infarctions or dilated cardiomyopathy. The measures of SVR and LVEDP were indirect, using the previously described formula. We have previously compared Doppler echocardiography with thermodilution techniques in multiple studies to assess the hemodynamic response to load alterations, vasodilators, and inotropic agents. On the basis of this experience and that of other groups, we believe that in short-term hemodynamic studies, Doppler echocardiographic results are as accurate as invasive hemodynamic data and provide significant information regarding directional changes.


