Additive Beneficial Effects of Losartan Combined With Simvastatin in the Treatment of Hypercholesterolemic, Hypertensive Patients

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Background—Biological mechanisms underlying statin and angiotensin II type 1 receptor blocker therapies differ. Therefore, we compared vascular and metabolic responses to these therapies either alone or in combination in hypercholesterolemic, hypertensive patients.

Methods and Results—This was a randomized, double-blind, placebo-controlled crossover trial with 3 treatment arms (each 2 months) and 2 washout periods (each 2 months). Forty-seven hypertensive, hypercholesterolemic patients were given simvastatin 20 mg and placebo, simvastatin 20 mg and losartan 100 mg, or losartan 100 mg and placebo daily during each 2-month treatment period. Losartan alone or combined therapy significantly reduced blood pressure compared with simvastatin alone. Compared with losartan alone, simvastatin alone or combined therapy significantly changed lipoproteins. All 3 treatment arms significantly improved flow-mediated dilator response to hyperemia and decreased plasma malondialdehyde and monocyte chemoattractant protein-1 levels relative to baseline measurements. However, these parameters were changed to a greater extent with combined therapy compared with simvastatin or losartan alone (both $P<0.001$ and $P=0.030$ for monocyte chemoattractant protein-1 by ANOVA). Combined therapy or losartan alone significantly increased plasma adiponectin levels and insulin sensitivity (determined by QUICKI) relative to baseline measurements. These changes were significantly greater than in the group treated with simvastatin alone ($P<0.001$ for adiponectin, $P=0.029$ for QUICKI by ANOVA).

Conclusions—Simvastatin combined with losartan improves endothelial function and reduces inflammatory markers to a greater extent than monotherapy with either drug in hypercholesterolemic, hypertensive patients. (Circulation. 2004;110:3687-3692.)

Key Words: angiotensin - endothelium - hypercholesterolemia - hypertension - insulin

Hypercholesterolemia and hypertension are major public health problems that are frequently treated with statins and angiotensin II type 1 (AT1) receptor blockers, respectively. Although the mechanisms of action for these 2 classes of drugs differ, both classes have beneficial effects on the vasculature. Indeed, large-scale clinical studies have demonstrated that simvastatin, an HMG-CoA reductase inhibitor, and losartan, an AT1 receptor blocker, prevent and retard the progression of coronary heart disease.1,2 Hypertension and coronary heart disease are frequently associated with insulin resistance and disorders of metabolic homeostasis such as obesity and type II diabetes. The endothelial dysfunction associated with cardiovascular diseases may contribute to insulin resistance and the pathophysiology of diabetes and its vascular complications.3 In fact, large-scale clinical studies have demonstrated that simvastatin and losartan reduce the onset of new diabetes.1,4 The mechanisms of this benefit may relate to the ability of these therapies to reduce insulin resistance.5 Moreover, it is possible that simvastatin combined with losartan therapy may have additional vascular benefits that are greater than those observed for either drug alone.

Statins reduce LDL cholesterol. In addition, they improve endothelial function via stimulation of nitric oxide (NO) synthase activity and mediate antioxidant effects that result in enhanced NO bioactivity.5,7 AT1 receptor blockers also improve endothelial function.8,9 This may be due in part to diminished intracellular production of superoxide anions via reduced activity of angiotensin II–dependent oxidases.10 Inhibition of the production of superoxide anions may limit oxidation of LDL and contribute to increased NO bioactivity by limiting oxidative degradation of NO.7 Thus, AT1 receptor
TABLE 1. Baseline Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57 ± 2</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>20:27</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.2 ± 0.5</td>
</tr>
<tr>
<td>Risk factors, n (%)</td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>11 (23)</td>
</tr>
<tr>
<td>β-Adrenergic blockers</td>
<td>10 (21)</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>6 (13)</td>
</tr>
</tbody>
</table>

BMI indicates body mass index. Values are expressed as mean ± SEM when appropriate. n = 47.

blockers may inhibit both LDL oxidation and atherosclerosis.

The endothelial dysfunction associated with diabetes, obesity, metabolic syndrome, and other insulin-resistant states is characterized by impaired NO release from endothelium. Thus, improvement in endothelial function is predicted to enhance insulin sensitivity, and this may be a mechanism by which simvastatin and losartan decrease the incidence of new-onset diabetes. Adiponectin is one of a number of proteins secreted by adipose cells that may couple regulation of insulin sensitivity with energy metabolism and serve to link obesity with insulin resistance. In humans, plasma levels of adiponectin are negatively correlated with adiposity, and decreased plasma adiponectin levels are observed in patients with diabetes and those with coronary artery disease. Thus, decreased levels of adiponectin may play a key role in the development of insulin resistance. In addition, adiponectin also possesses antiatherogenic properties.

Because the impact of simvastatin and losartan therapies on NO bioactivity and its subsequent effects on oxidant stress, inflammation, endothelial function, and insulin resistance may differ, we hypothesized that combined therapy may have additive beneficial effects that are greater than those observed with either simvastatin or losartan therapy alone in hypercholesterolemic, hypertensive patients.

Methods
Study Population and Design
Fifty hypercholesterolemic, hypertensive patients (LDL cholesterol levels ≥100 mg/dL) participated in this study. We defined hypertension as systolic and diastolic blood pressure ≥140 or ≥90 mm Hg, respectively. We excluded patients with severe hypertension, unstable angina, or acute myocardial infarction. To minimize acute side effects to losartan, study medication was titrated from 50 to 100 mg upward over a 2-week period if no hypotension (systolic blood pressure <100 mm Hg) was noted. At the end of this time, participants were receiving either placebo or losartan 100 mg/d. Of 50 patients, 47 tolerated losartan 100 mg with regard to maintaining systolic blood pressure >100 mm Hg for 3 hours after drug administration and experienced no adverse effects from therapy. One patient was hypotensive, and the other 2 patients suffered from dry cough. Thus, a total of 47 patients’ data were analyzed. The clinical characteristics of these patients are summarized in Table 1. Patients were randomly assigned to one of the 3 treatments: simvastatin 20 mg and placebo, simvastatin 20 mg and losartan 100 mg, or losartan 100 mg and placebo daily during 2 months. This was a randomized, double-blind, placebo-controlled study with 3 treatment arms (each 2 months) and crossover with 2 washout periods (each 2 months). The patients were seen at 14-day intervals (or more frequently) during the study. Calcium channel or β-adrenergic blockers were withheld for ≥48 hours before the study to avoid the effects of these drugs. The Gil Hospital Institute Review Board approved the study, and all participants gave written, informed consent.

Laboratory Assays
Blood samples for laboratory assays were obtained at approximately 8 AM after patients fasted overnight before and at the end of each 2-month treatment period. These samples were immediately coded so that investigators performing laboratory assays were blinded to subject identity or study sequence. Assays for lipids, glucose, and plasma malondialdehyde (MDA), monocyte chemoattractant protein (MCP)-1, and adiponectin were performed in duplicate by ELISA (BIOXYTECH LPO-586, OxisResearch, and R&D Systems, Inc) and assays for high-sensitivity C-reactive protein (hsCRP) levels by latex agglutination (CRP-Latex(II), Denka-Seiken) as previously described. Assays for plasma insulin levels were performed in duplicate by immunoradiometric assay (INSULIN-RIA BEAD II, Abbott Japan). The interassay and intra-assay coefficients of variation were <6%. Quantitative Insulin-Sensitivity Check Index (QUICKI), a surrogate index of insulin sensitivity, was calculated as follows (insulin is expressed in μU/mL and glucose in mg/dL): QUICKI = 1/[log(insulin) + log(glucose)].

Vascular Studies
Imaging studies of the right brachial artery were performed with an ATL HDI 3000 ultrasound machine (Bothell) equipped with a 10-MHz linear-array transducer based on a previously published technique. Measurements were performed by 2 independent investigators (S.H.H. and W.-J.C.) blinded to the subject’s identity and medication status. Measurements of maximum diameter and percent flow-mediated dilation were made in 10 studies selected at random. The interobserver and intraobserver variabilities for repeated measurement of maximum diameter were 0.01 ± 0.06 and 0.008 ± 0.05 mm, respectively. The interobserver and intraobserver variabilities for repeated measurement of percent flow-mediated dilation were 0.12 ± 0.31% and 0.10 ± 1.29%, respectively.

Statistical Analysis
Data are expressed as mean ± SEM or median (25% to 75% range). After testing data for normality, we used Student’s paired t or Wilcoxon signed-rank test to compare values before and after each treatment and the relative changes in values in response to treatment, as reported in Tables 2 and 3. The effects of the 3 therapies on vascular function, markers of oxidant stress and inflammation, and insulin sensitivity relative to baseline values were analyzed by 1-way repeated-measures ANOVA or Friedman’s repeated ANOVA on ranks. After demonstration of significant differences among therapies by ANOVA, post hoc comparisons between treatment pairs were made by use of the Student-Newman-Keuls multiple comparison procedures. Pearson’s correlation coefficient analysis was used to assess associations between measured parameters. We calculated that 30 subjects would provide 80% power for detecting a difference of absolute increase, ≥2.1% flow-mediated dilation of the brachial artery between baseline and simvastatin, with α = 0.05 based on our previous studies. The comparison of endothelium-dependent dilation among the 3 treatment schemes was prospectively designated as the primary end point of the study. All other comparisons were considered secondary. A value of P < 0.05 was considered statistically significant.

Results
When baseline values before each treatment period were compared among the 3 treatment arms, no significant differences were noted in any of the parameters measured (Tables 2 and 3). To rule out the possibility of a carryover effect from one treatment period to the next, we compared baseline values before the first treatment period to those before the
TABLE 3. Effects of Simvastatin, Combined Therapy, and Losartan on Adiponectin Levels and Insulin Resistance in Hypercholesterolemic, Hypertensive Patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Simvastatin</th>
<th>Simvastatin + Losartan</th>
<th>Losartan</th>
<th>ANOVA</th>
<th>S/C</th>
<th>S/L</th>
<th>C/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP, μM/mL</td>
<td>4.5 (3.4–7.0)</td>
<td>4.5 (2.9–6.0)</td>
<td>4.6 (3.3–6.4)</td>
<td>5.0 (4.0–7.3)</td>
<td>4.2 (3.5–6.2)</td>
<td>5.3 (3.8–6.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin, μU/mL</td>
<td>2.67 ± 0.29</td>
<td>3.06 ± 0.38</td>
<td>2.56 ± 0.26</td>
<td>2.40 ± 0.28</td>
<td>2.79 ± 0.26</td>
<td>2.49 ± 0.28</td>
<td>0.041</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>82 ± 2</td>
<td>83 ± 2</td>
<td>82 ± 2</td>
<td>79 ± 2</td>
<td>83 ± 2</td>
<td>80 ± 2</td>
<td>0.348</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.475 ± 0.016</td>
<td>0.458 ± 0.013</td>
<td>0.467 ± 0.012</td>
<td>0.497 ± 0.114*</td>
<td>0.456 ± 0.012</td>
<td>0.493 ± 0.015*</td>
<td>0.029</td>
</tr>
</tbody>
</table>

S indicates simvastatin; C, combined therapy; L, losartan; and ADP, adenosine diphosphate. Data are expressed as mean ± SEM or median (25th to 75th percentiles). There were no significant differences among each baseline value.

Effects of Therapies on Vasomotor Function and MDA

Simvastatin, combined therapy, and losartan significantly improved the percent flow-mediated dilator response to hyperemia relative to baseline measurements by 38 ± 4%, 68 ± 4%, and 31 ± 3%, respectively (all P < 0.001); however, combined therapy significantly improved this response more than simvastatin or losartan alone (P < 0.001 by ANOVA; Figure 1 and Table 2). The brachial artery dilator response to nitroglycerin was similar for all therapies and was not significantly changed from baseline values. Simvastatin, combined therapy, and losartan significantly decreased the plasma MDA levels relative to baseline measurements by 11 ± 3% (P < 0.001), 23 ± 4% (P < 0.001), and 5 ± 3% (P = 0.040), respectively; however, combined therapy significantly reduced MDA levels more than simvastatin or losartan alone (P < 0.001 by ANOVA; Figure 2 and Table 2).

Effects of Therapies on Markers of Inflammation

Simvastatin, combined therapy, and losartan significantly decreased plasma MCP-1 levels relative to baseline measurements by 7 ± 3% (P = 0.003), 15 ± 3% (P < 0.001), and 5 ± 4% (P = 0.048), respectively; however, combined therapy significantly decreased MCP-1 levels more than simvastatin or
Simvastatin, combined therapy, and losartan significantly lowered plasma hsCRP levels relative to baseline measurements from 0.85 to 0.80 mg/L ($P<0.042$), 0.85 to 0.65 mg/L ($P<0.002$), and 0.85 to 0.80 mg/L ($P<0.042$), respectively; however, the magnitude of reduction among these 3 therapies was not statistically significant ($P<0.146$ by ANOVA).

**Effects of Therapies on Adiponectin and Insulin Resistance**

There were significant inverse correlations between body mass index and baseline plasma adiponectin levels ($r=-0.332$, $P=0.023$ before simvastatin; $r=-0.328$, $P=0.024$ before combined therapy; $r=-0.292$, $P=0.046$ before losartan). There were significant inverse correlations between baseline adiponectin levels and baseline triglyceride levels ($r=-0.351$, $P=0.016$ before simvastatin; $r=-0.325$, $P=0.026$ before combined therapy; $r=-0.342$, $P=0.019$ before losartan). There were significant correlations between baseline adiponectin levels and baseline HDL cholesterol levels ($r=0.401$, $P=0.005$ before simvastatin; $r=0.399$, $P=0.006$ before combined therapy; $r=0.303$, $P=0.039$ before losartan). Combined therapy and losartan alone significantly increased the plasma adiponectin levels relative to baseline measurements from 4.63 to 5.02 ($P<0.001$) and 4.19 to 5.27 ($P=0.002$), respectively. These increases were significantly greater than those observed with simvastatin alone ($P<0.001$ by ANOVA; Figure 4 and Table 3). The 3 therapies did not have significantly different baseline insulin and glucose levels. However, the magnitude of reduction of insulin with combined therapy or losartan alone was significantly greater than with simvastatin alone ($P=0.041$ by ANOVA; Table 3). Combined therapy or losartan alone significantly increased QUICKI relative to baseline measurements by $7\%$ ($P=0.032$) and $7\%$ ($P=0.042$), respectively. These increases were significantly greater than those observed with simvastatin alone ($P=0.029$ by ANOVA; Figure 5 and Table 3). There were correlations between percent changes in adiponectin levels and percent changes in QUICKI ($r=0.245$, $P=0.097$ after simvastatin; $r=0.316$, $P=0.030$ after combined therapy; $r=0.433$, $P=0.002$ after losartan). There were inverse correlations between percent changes in adiponectin levels and percent changes in insulin ($r=0.171$, $P=0.251$ after simvastatin; $r=0.352$, $P=0.015$ after combined therapy; $r=0.367$, $P=0.011$ after losartan).

We investigated whether losartan-induced changes in the percent flow-mediated dilator response to hyperemia, serological markers of oxidant stress and inflammation, and insulin resistance were mediated by a reduction in systolic or diastolic blood pressure.
diastolic blood pressure. There were no significant correlations between these changes and reduction of systolic blood pressure ($-0.134 \leq r = 0.077$) or between these changes and reduction of diastolic blood pressure ($-0.295 \leq r = 0.172$). After combined therapy, improvement in flow-mediated dilation correlated with changes in MDA levels ($r = -0.422$ and $P = 0.003$), MCP-1 levels ($r = 0.189$ and $P = 0.204$), hsCRP levels ($r = -0.137$ and $P = 0.357$), adiponectin levels ($r = 0.420$ and $P = 0.003$), QUICKI ($r = 0.258$ and $P = 0.080$), and insulin levels ($r = -0.251$ and $P = 0.089$).

Discussion

In our hypercholesterolemic, hypertensive cohort, simvastatin therapy alone significantly improved the lipid profile, whereas losartan therapy alone significantly lowered blood pressure as expected. Comparable beneficial effects on both lipids and blood pressure were observed with combination therapy. We reasoned that distinct biological actions of simvastatin and losartan therapies on lipoproteins and the angiotensin system may improve endothelium-dependent vascular function by different mechanisms. Indeed, although monotherapy with simvastatin or losartan significantly improved endothelial function and inflammatory markers (assessed by flow-mediated dilation, MDA levels, CRP levels, and MCP-1 levels), combined therapy had additional substantial and significant beneficial effects on these parameters over those seen with monotherapy for either drug, which may explain the observations of a recent clinical trial.21

The additional beneficial effects of combined simvastatin/losartan therapy may be the result of several interacting mechanisms. For example, angiotensin II is very potent endogenous vasoconstrictor, whereas LDL induces upregulation of the AT1 receptor.22 Indeed, hypercholesterolemic rabbits display enhanced vascular expression of AT1 receptors that mediate increased activity of angiotensin II.23 Furthermore, the effect of statins to reverse the elevated blood pressure response to angiotensin II infusion is accompanied by downregulated AT1 receptor density.24,25 Angiotensin II promotes superoxide anion generation and endothelial dysfunction.8,26 CRP upregulates AT1 receptors in vascular smooth muscle cells, and these effects are attenuated by losartan.27 The additive beneficial effects of combined therapy in the present study are consistent with experimental and clinical studies.21,28

Losartan therapy alone resulted in significant elevation of adiponectin levels, decreased insulin levels, and increased insulin sensitivity (assessed by QUICKI). The present study is the first report demonstrating that losartan therapy can increase adiponectin levels. Adiponectin is an adipose-derived factor that augments and mimics metabolic actions of insulin. Increasing adiponectin levels would be predicted to improve both insulin sensitivity and endothelial function by multiple mechanisms. Regulation of metabolic homeostasis and hemodynamic homeostasis may be coupled by vascular actions of insulin to stimulate production of NO.16 Thus, improvements in endothelial function may increase insulin sensitivity, whereas increased insulin sensitivity may improve endothelial function.12 Interestingly, in contrast to the effects of combination therapy on flow-mediated dilation, MDA, CRP, and MCP-1, the beneficial effects of losartan therapy on adiponectin levels, insulin levels, and insulin sensitivity did not increase further with combination therapy. This finding suggests that improving endothelial function per se (as reflected by flow-mediated dilation) may not completely explain the effects of losartan or combined therapy to improve insulin sensitivity. In other words, there may be additional mechanisms for losartan or combined therapy to improve insulin sensitivity that are independent of endothelial function, eg, direct effects of losartan on glucose insulin–stimulated glucose uptake or promotion of adipogenic differentiation of preadipocytes29 or induction of peroxisome proliferator–activated receptor-$\gamma$ activity promoting differentiation in adipocytes.30 Effects of losartan or combined therapy to increase adiponectin levels may in part mediate improved insulin sensitivity, which is supported by the significant correlation shown in the present study. On the other hand, combined therapy may reduce insulin resistance by multiple mechanisms such as lipoprotein changes and reduced oxidant stress that also contribute to NO bioavailability. The effects of losartan or combined therapy on flow-mediated dilation, oxidant stress and inflammation markers, and insulin resistance were independent of blood pressure changes and consistent with recent randomized clinical trials.2,31 Likewise, several studies suggest a hypothesis that the effects of AT1 receptor blockers to improve endothelial function are due to other factors in addition to a reduction in blood pressure.32,33

Metabolic syndrome is associated with atherosclerotic disease. Patients with metabolic syndrome make up one of the largest groups of individuals with both hyperlipidemia and hypertension. Obesity is one of the most common causes of cardiovascular disease. In the present study, more than half of the subjects were overweight. We observed that plasma levels of adiponectin were significantly inversely correlated with body mass index. We also observed significant correlations between baseline adiponectin levels and baseline HDL cholesterol or triglyceride levels. Thus, our study may have implications for the treatment of patients with metabolic syndrome.

In summary, our study suggests that combination therapy with simvastatin and losartan has beneficial additive effects
on endothelial function and inflammatory markers. This may be due to combined effects of the respective monotherapies to improve lipid profile, blood pressure, adiponectin levels, and insulin sensitivity. The additive beneficial effects of combined therapy are predicted to reduce cardiovascular events in hypercholesterolemic, hypertensive patients more than monotherapy with either drug alone.

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References

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