Simvastatin Combined With Ramipril Treatment in Hypercholesterolemic Patients

Kwang Kon Koh, Ji Won Son, Jeong Yeal Ahn, Dae Sung Kim, Dong Kyu Jin, Hyung Sik Kim, Seung Hwan Han, Yiel-Hea Seo, Wook-Jin Chung, Woong Chol Kang, Eak Kyun Shin

Abstract—Mechanisms underlying biological effects of statin and angiotensin-converting enzyme inhibitor therapies differ. Thus, we studied vascular responses to combination therapy in hypercholesterolemic patients. A randomized, double-blind, placebo-controlled, crossover trial was conducted with 50 hypercholesterolemic patients with simvastatin and either placebo or ramipril (study I) and in 45 hypercholesterolemic diabetic patients with simvastatin or ramipril with placebo or simvastatin combined with ramipril (study II) for 2 months with 2 months washout. In study I simvastatin combined with ramipril significantly reduced blood pressure after 2 months. Simvastatin alone or combined with ramipril significantly changed lipoproteins, improved percent flow-mediated dilator response to hyperemia by 30±5% and 53±6%, respectively (P<0.001), and reduced plasma levels of malondialdehyde by 4±7% (P=0.026) and 25±4% (P<0.001), respectively. Monocyte chemotactant protein-1 levels decreased by 3±3% and 12±2%, respectively (P=0.049 and P=0.001, respectively), C-reactive protein levels changed by 0% and 18%, respectively (P=0.036 and P<0.001, respectively), and plasminogen activator inhibitor-1 antigen levels changed by −7±7% and 17±5%, respectively (P=0.828 and P<0.001, respectively). In study II ramipril alone did not significantly change lipoproteins and C-reactive protein levels, however, simvastatin combined with ramipril significantly changed lipoproteins and C-reactive protein levels more than ramipril alone (P<0.001 and P=0.048 by ANOVA, respectively). Ramipril alone or simvastatin combined with ramipril significantly improved the percent flow-mediated dilator response to hyperemia (both P<0.001), however, simvastatin combined with ramipril showed significantly more improvement than ramipril alone (P<0.001 by ANOVA). Simvastatin combined with ramipril significantly improved endothelium-dependent vasodilation and fibrinolysis potential and reduced plasma levels of oxidant stress and inflammation markers in hypercholesterolemic patients. (Hypertension. 2004;44:180-185.)

Key Words: angiotensin-converting enzyme ◼ atherosclerosis ◼ endothelial growth factors ◼ hypercholesterolemia ◼ blood pressure

Large-scale clinical studies demonstrate that statins and angiotensin-converting enzyme (ACE) inhibitor prevent or retard the progression of coronary heart disease.1,2 The mechanisms of this apparent benefit of these therapies may include vascular effects. Statins reduce LDL cholesterol and improve endothelial function, consistent with enhanced NO bioactivity.3–5 ACE inhibition also improves endothelial function.6,7 A potential mechanism of this effect is augmented NO bioactivity via diminished bradykinin degradation by ACE with activation of endothelial B2 kinin receptors and stimulation of NO synthase activity.8 Alternatively, ACE inhibition may diminish intracellular production of superoxide anions via reduced activity of angiotensin II–dependent oxidases in the endothelium and vascular smooth muscle,9,10 thus protecting NO from oxidant degradation to biologically inert or toxic molecules.3,11 Inhibition of the production of superoxide anions may also limit the oxidation of LDL, thus contributing to increased NO bioactivity by enhancing NO synthesis and limiting oxidative degradation of NO.3,11 Indeed, ACE inhibitors inhibit LDL oxidation and attenuate atherosclerosis.12

Recent studies have shown that LDL induces the expression of angiotensin II type-1 (AT1) receptor upregulation and that hypercholesterolemic rabbits display enhanced vascular expression of AT1 receptors.13,14 Of interest, statins reverse the elevated blood pressure response to angiotensin II infusion and downregulate AT1 receptor density.15 Recent experimental studies have confirmed that angiotensin II accelerates the development of atherosclerosis.16,17 These studies suggest that angiotensin II promotes superoxide anion generation and endothelial dysfunction. This effect is mediated by AT1.
pressure tolerated 10 mg ramipril with regard to maintaining systolic blood pressure <100 mm Hg) was noted. At the end of this time participants were receiving either placebo or ramipril per day. Fifty among 53 patients included calcium-channel blockers and long-acting nitrates, were withheld for ≥24 hours before the study. The study was approved by the Gil Hospital Institute Review Board, and all participants gave written, informed consent.

Because we did not investigate the vascular effects of 10 mg ramipril alone, we performed another study to compare the vascular effects of 20 mg simvastatin and placebo, 20 mg simvastatin combined with 10 mg ramipril, and 10 mg ramipril and placebo under the same protocol in hypercholesterolemic, type 2 diabetic patients. This study design was randomized, double-blind, and placebo-controlled, with 3 treatment arms (each 2 months) and crossover with 2 washout periods (each 2 months). 45 patients finished 3 treatment arms. 14 of the 45 patients participated in the original study.

### Laboratory Assays
Blood samples for laboratory assays were obtained at approximately 8:00 AM following overnight fasting before and at the end of each treatment period for 2 months and immediately coded so that investigators performing laboratory assays were blinded to subject identity or study sequence. Assays for lipids, plasma nitrate (using the Griess reaction), malondialdehyde (MDA), monocyte chemottractant protein (MCP)-1, and PAI-1 antigen were performed in duplicate by enzyme-linked immunosorbent assay (R&D Systems, Bioxytech LPO-586, OxisResearch, or Biopool for PAI-1 antigen) as previously described.4,7,21,22 C-reactive protein (CRP) levels were determined with an immunonephelometry system according to methods described by the manufacturer (rate nephelometry; Immage, Beckman Coulter) as previously described.23,24

### Vascular Studies
Imaging studies of the right brachial artery were performed using a ATL HDI 3000 ultrasound machine equipped with a 10 MHz linear-array transducer, based on a previously published technique.4,7,21,22 Measurements were performed by 2 independent investigators (D.K.J. and H.S.K.) blinded to the subject’s identity and medication status.

### Statistical Analysis
Data are expressed as mean±SEM or median (range 25% to 75%). After testing data for normality, we used Student 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 for simvastatin + placebo versus simvastatin + ramipril. The effects of simvastatin + placebo, ramipril + placebo, and simvastatin + ramipril were analyzed by 1-way repeated measures ANOVA or Friedman 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 or Spearman correlation coefficient analysis was used to assess associations between measured parameters. P<0.05 was considered to be statistically significant.

### Results
No significant differences were noted between baseline values for each treatment group (Tables 2 and 3). To rule out the possibility of a carryover effect from one treatment period to the next treatment period, we compared baseline values before the first treatment period to those before the second and third treatment periods. No significant differences were found with this analysis.

### Simvastatin + Placebo Versus Simvastatin + Ramipril Study

#### Effects of Therapies on Blood Pressure and Lipids
Resting heart rate was similar after each treatment. Simvastatin alone did not reduce systolic and diastolic blood
pressure after 2-month administration. Simvastatin combined with ramipril significantly reduced systolic and diastolic blood pressure after 2-month administration compared with baseline, and these reductions were greater than simvastatin alone. Simvastatin alone or combined with ramipril significantly lowered total cholesterol (both P<0.001) and triglyceride levels (P=0.003 and P<0.001, respectively), lowered LDL cholesterol (both P<0.001) and apolipoprotein B levels (both P<0.001), and increased HDL cholesterol (P=0.742 and P=0.260, respectively) and apolipoprotein A-I levels (P=0.016 and P=0.049, respectively). However, there were no significant differences between each treatment (Table 2).

-effects of Therapies on Vasomotor Function, Nitrate, and MDA

Simvastatin alone or combined with ramipril significantly improved the percent flow-mediated dilator response to hyperemia relative to baseline measurements by 30±5% and by 53±6%, respectively (both P<0.001). Of note, simvastatin combined with ramipril resulted in a significant improvement over simvastatin alone (P<0.001; Figure 1; Table 2). The brachial artery dilator response to nitroglycerin between each therapy was not significantly increased compared with respective baseline values for either therapy. Simvastatin alone or combined with ramipril decreased the plasma-nitrate

### TABLE 2. Effects of Simvastatin and Ramipril on Lipid Levels and Endothelial Function in 50 Hypercholesterolemic Patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline 1</th>
<th>Simvastatin (S)</th>
<th>Baseline 2</th>
<th>Combination (C)</th>
<th>P (S vs C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>71±2</td>
<td>71±2 (0±2)</td>
<td>72±2</td>
<td>71±2 (−1±1)</td>
<td>0.819</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>132±2</td>
<td>131±3 (0±2)</td>
<td>134±3</td>
<td>126±3 (−5±2)‡</td>
<td>0.048</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>74±1</td>
<td>75±1 (2±2)</td>
<td>77±2</td>
<td>72±1 (−5±2)‡</td>
<td>0.021</td>
</tr>
<tr>
<td>Lipids, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>250±4</td>
<td>173±5 (−31±2)‡</td>
<td>247±6</td>
<td>178±5 (−28±1)‡</td>
<td>0.164</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>183±16</td>
<td>143±8 (−11±5)†</td>
<td>202±14</td>
<td>148±10 (−20±4)‡</td>
<td>0.145</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>164±4</td>
<td>95±4 (−42±2)‡</td>
<td>159±5</td>
<td>99±4 (−38±2)‡</td>
<td>0.211</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>131±4</td>
<td>89±3 (−31±2)‡</td>
<td>129±4</td>
<td>92±3 (−27±3)‡</td>
<td>0.150</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>51±1</td>
<td>51±2 (3±3)</td>
<td>51±2</td>
<td>56±4 (13±9)</td>
<td>0.261</td>
</tr>
<tr>
<td>Apolipoprotein A-I</td>
<td>144±3</td>
<td>154±4 (8±3)*</td>
<td>146±3</td>
<td>153±4 (6±3)*</td>
<td>0.826</td>
</tr>
<tr>
<td>Vasomotor function, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow-mediated dilation</td>
<td>4.81±0.24</td>
<td>6.02±0.29 (30±5)‡</td>
<td>4.56±0.22</td>
<td>6.58±0.25 (53±6)‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nitroglycerin dilation</td>
<td>12.55±0.65</td>
<td>13.01±0.60 (10±5)‡</td>
<td>12.36±0.55</td>
<td>12.83±0.57 (6±5)</td>
<td>0.379</td>
</tr>
<tr>
<td>Nitrate, μmol/L</td>
<td>92±7</td>
<td>83±6 (7±8)</td>
<td>89±6</td>
<td>74±5 (−8±6)*</td>
<td>0.115</td>
</tr>
<tr>
<td>Malondialdehyde, μmol/L</td>
<td>1.36±0.08</td>
<td>1.17±0.07 (−4±7)*</td>
<td>1.45±0.09</td>
<td>1.01±0.07 (−25±4)‡</td>
<td>0.009</td>
</tr>
<tr>
<td>Cytokines and hemostasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1, pg/mL</td>
<td>194±8</td>
<td>178±5 (−3±3)*</td>
<td>202±8</td>
<td>174±6 (−12±2)‡</td>
<td>0.015</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.9 (1.1−3.7)</td>
<td>1.1 (1.1−1.8) (6%)*</td>
<td>2.4 (1.1−4.1)</td>
<td>1.1 (1.1−2.2) (18%)‡</td>
<td>0.150</td>
</tr>
<tr>
<td>PAI-1, ng/mL</td>
<td>64±4</td>
<td>63±4 (3±7)</td>
<td>68±4</td>
<td>53±3 (−17±5)‡</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Data are expressed as means±SEM or median (25th percentile–75th percentile and median percent changes from respective baseline) for CRP. The numbers in parenthesis represent means±SEM of percent changes from respective baseline.

### TABLE 3. Vascular Effects of Simvastatin, Combination, and Ramipril in 45 Hypercholesterolemic Diabetic Patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Simvastatin + Placebo</th>
<th>Simvastatin + Ramipril</th>
<th>Ramipril + Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids, mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>228±6</td>
<td>162±5 (−29±1)*</td>
<td>227±7</td>
<td>223±8</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>237±28</td>
<td>172±15 (−20±4)*</td>
<td>228±19</td>
<td>214±19</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>135±7</td>
<td>79±5 (−41±2)</td>
<td>135±6</td>
<td>133±7</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>124±4</td>
<td>84±3 (−31±2)*</td>
<td>126±4</td>
<td>117±4</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>46±2</td>
<td>45±2 (0±3)</td>
<td>47±2</td>
<td>47±2</td>
</tr>
<tr>
<td>Apolipoprotein A-I</td>
<td>147±3</td>
<td>150±3 (3±2)</td>
<td>151±4</td>
<td>145±4</td>
</tr>
<tr>
<td>Flow-mediated dilation, %</td>
<td>4.11±0.17</td>
<td>5.80±0.21 (46±5)*</td>
<td>4.11±0.19</td>
<td>4.17±0.16</td>
</tr>
<tr>
<td>Nitroglycerin-mediated dilatation, %</td>
<td>12.41±0.60</td>
<td>12.82±0.64 (6±4)</td>
<td>13.10±0.57</td>
<td>12.98±0.56</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>1.7 (1.1–3.3)</td>
<td>1.4 (1.1–2.7) (−4%)</td>
<td>2.2 (1.1–4.1)</td>
<td>1.6 (1.1–3.4)</td>
</tr>
</tbody>
</table>

Data are expressed as means±SEM or median (25th percentile–75th percentile and median percent changes from respective baseline) for CRP. The numbers in parenthesis represent means±SEM of percent changes from respective baseline.

There were no significant differences among each baseline values.

*P<0.001 for comparison with each baseline value.
levels relative to baseline measurements by -7±8% and by 8±6%, respectively (P=0.289 and P=0.015, respectively). However, there was no significant difference between treatments (P=0.115). Simvastatin alone or combined with ramipril significantly improved the percent flow-mediated dilator response to hyperemia relative to baseline measurements. However, the effect of simvastatin combined with ramipril was significantly greater than simvastatin alone. Mean values are identified by □.

**Effects of Therapies on Markers of Inflammation and Hemostasis**

**Cytokines**

Simvastatin alone or combined with ramipril lowered plasma levels of MCP-1 relative to baseline measurements by 3±3% and by 12±2%, respectively (P=0.049 and P=0.001, respectively; Table 2). Simvastatin combined with ramipril had a significantly larger effect than simvastatin alone (P=0.015). Simvastatin alone or combined with ramipril significantly lowered serum levels of CRP relative to baseline measurements by 0% and by 18%, respectively (P=0.036 and P<0.001, respectively). Simvastatin combined with ramipril did not have a significantly greater effect than simvastatin alone (P=0.150).

**Hemostasis**

Simvastatin combined with ramipril lowered plasma levels of PAI-1 antigen relative to baseline by 17±5% (P<0.001), and this effect of simvastatin combined with ramipril was significantly different from simvastatin alone (P=0.003; Figure 2; Table 2).

We investigated whether added ramipril-induced changes in the percent flow-mediated dilator response to hyperemia and serological markers of oxidant stress, inflammation, and fibrinolysis were mediated by reduction of systolic or diastolic blood pressure. There were no significant correlations between these changes and reduction of systolic blood pressure (-0.148≤r≤0.110) and between these changes and reduction of diastolic blood pressure (-0.216≤r≤0.138). Improvement in flow-mediated dilation did not correlate with changes in plasma nitrate levels (r=0.074 and r=0.127), MDA levels (r=−0.051 and r=−0.095), MCP-1 levels (r=−0.026 and r=0.039), CRP levels (r=0.294 and r=0.023), and PAI-1 levels (r=0.170 and r=0.227) after simvastatin alone or combined with ramipril. Furthermore, to identify a mechanism for the regulation of CRP and MCP-1 levels, we assessed correlations between CRP levels and MCP-1 levels. There were no significant correlations between CRP levels and MCP-1 levels (r=−0.162 and r=−0.218).

**Simvastatin+Placebo, Simvastatin+Ramipril, and Ramipril+Placebo Study**

We observed that ramipril alone did not significantly change lipoproteins and CRP levels relative to baseline measurements, however, simvastatin combined with ramipril significantly changed lipoproteins and CRP levels relative to baseline measurements (both P<0.001; Table 3). Furthermore, simvastatin combined with ramipril significantly changed lipoproteins and CRP levels more than ramipril alone (P<0.001 and P=0.048 by ANOVA, respectively; Table 3). Ramipril alone or simvastatin combined with ramipril significantly improved the percent flow-mediated dilator response to hyperemia relative to baseline measurements (both P<0.001), however, simvastatin combined with ramipril significantly improved more than ramipril alone (P<0.001 by ANOVA; Table 3).

**Discussion**

Simvastatin and ramipril have different action mechanisms to affect vascular function and endothelium-dependent vasodilator responsiveness through modulating lipoprotein and angiotensin physiology. Therefore, we reasoned that combined therapy may have additive or synergistic beneficial effects in
subjects with hypercholesterolemia. Indeed, whereas either monotherapy or combined therapy significantly improved the percent flow-mediated dilator response of the brachial artery, the effect of simvastatin combined with ramipril was significantly greater than that of monotherapy, an effect consistent with enhanced release of NO.

LDL induces the expression of AT_1 receptor upregulation, and hypercholesterolemic rabbits display enhanced vascular expression of AT_1 receptors representing increased activity of angiotensin II.\textsuperscript{13,14} Of interest, statins reverse the elevated blood pressure response to angiotensin II infusion and down-regulate AT_1 receptor density.\textsuperscript{15} Therefore, simvastatin combined with ramipril may reduce LDL cholesterol levels and angiotensin II expression, which would be predicted to improve flow-mediated dilation. Indeed, consistent with other studies,\textsuperscript{16,25} we observed that simvastatin combined with ramipril significantly improved the percent flow-mediated dilator response to hyperemia to greater extent than simvastatin or ramipril alone. One recent study reported no additional benefits on coronary atherosclerosis progression measured by quantitative coronary angiogram when simvastatin was combined with enalapril.\textsuperscript{26} However, this study was done in normocholesterolemic patients, and the diameter change of coronary artery may not reflect important biological changes in the cell. Indeed, careful analysis of that study shows that when compared with placebo patients, fewer patients with enalapril experienced the combined end point of death/myocardial infarction/stroke less often despite no benefits on the diameter change of coronary artery.

Consistent with our previous studies,\textsuperscript{4,7} plasma nitrate levels, reflecting luminal release of NO,\textsuperscript{27} were marginally and significantly reduced with either simvastatin alone or in combination with ramipril. Reduction in luminal release of NO after simvastatin alone or in combination with ramipril may indicate reduced synthesis of NO by constitutive NO synthase. This, in turn, may be a consequence of reduced NO degradation by oxidized lipoproteins, free radical molecules from the endothelium and from inflammatory cells,\textsuperscript{28} and decreased superoxide anions generated by angiotensin II–dependent nicotinamide adenine dinucleotide (phosphate) oxidades within the vascular wall.\textsuperscript{9,10} In the current study, we observed that simvastatin alone or combined with ramipril decreased the plasma MDA levels relative to baseline measurements whereas simvastatin combined with ramipril resulted in an even greater effect than simvastatin alone (consistent with an experimental study).\textsuperscript{29}

We observed that simvastatin combined with ramipril had a significantly greater effect to reduce MCP-1 levels than simvastatin alone. We hypothesized that simvastatin may reduce plasma MCP-1 levels via inactivation of NF-κB. This would be predicted to improve NO bioactivity as reflected in improved flow-mediated dilation. However, we did not observe significant inverse correlations between MCP-1 levels and flow-mediated dilatation. CRP is now considered to be an important inflammatory mediator as well as acute phase reactant. CRP upregulates AT_1 receptors in vascular smooth muscle cells, and these effects are attenuated by losartan.\textsuperscript{30} In the current study, we observed that simvastatin combined with ramipril significantly reduced CRP levels greater than ramipril alone.

Bourcier et al\textsuperscript{31} demonstrated that simvastatin reduces levels of PAI-1 antigen released from smooth muscle cells and endothelial cells. However, in hypercholesterolemic post-menopausal women,\textsuperscript{4} we did not observe reduction of PAI-1 by simvastatin. The reduction of PAI-1 in our present study is consistent with observation of increased PAI-1 levels in humans after infusion of angiotensin II.\textsuperscript{32} Of interest, we observed that simvastatin combined with ramipril significantly reduced PAI-1 antigen levels more than simvastatin alone.

**Perspectives**

Impaired endothelial vasodilation is associated with increased cardiovascular event rates. Furthermore, endothelial dysfunction and increased vascular oxidative stress predict the risk of cardiovascular event rates in patients with coronary artery disease.\textsuperscript{33,34} MCP-1, CRP, and PAI-1 are independent serological markers that predict cardiovascular events. We observed that simvastatin combined with ramipril improves endothelial function as reflected by improved flow-mediated dilation, improves fibrinolysis potential, and reduces oxidant stress and inflammatory markers. Accordingly, combined therapy is expected to be more effective to reduce the cardiovascular events than simvastatin or ramipril alone in hypercholesterolemic patients independent of effects to lower blood pressure.\textsuperscript{35}

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**References**


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