Simvastatin Treatment Is Associated With Improvement in Coronary Endothelial Function and Decreased Cytokine Activation in Patients After Heart Transplantation

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OBJECTIVES
This study was designed to assess the association between 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibition, coronary endothelial function and cytokine activation in heart transplant recipients without angiographically detectable disease.

BACKGROUND
Coronary endothelial dysfunction contributes to cardiac allograft vasculopathy. The vasoprotective effects of statins in heart transplant recipients may include restoration of endothelial function and suppression of allograft inflammatory activity.

METHODS
Heart transplant recipients (one to three years after heart transplant) were divided into three groups based on the total cholesterol levels: group 1 (n = 21), patients with a history of hypercholesterolemia adequately controlled with simvastatin; group 2 (n = 19), patients with hypercholesterolemia not adequately treated with simvastatin; and group 3 (n = 40), patients without hypercholesterolemia. Coronary vasomotor function and intimal thickness as well as coronary sinus and aortic cytokine concentrations (tumor necrosis factor [TNF]-α, interleukin [IL]-6 and soluble IL-2 receptor) were investigated. In a prospective one-year follow-up study, changes in coronary endothelial function and cytokine levels were compared between 11 hypercholesterolemic patients treated with simvastatin and 9 controls.

RESULTS
Epicardial and microvascular endothelial functions were better in groups 1 and 3 than they were in group 2 (p < 0.01 and p < 0.05). Transcardiac IL-6 and TNF-α gradients were significantly increased in groups 2 and 3 compared with group 1 (IL-6: p < 0.05; TNF-α: p < 0.01). Plaque areas were significantly increased in groups 1 and 2 (p < 0.05 vs. group 3), whereas lumen area was increased in group 2 compared with group 1 (p < 0.05), demonstrating adaptive vascular remodeling. In patients treated with simvastatin, coronary endothelial function and cardiac cytokine activity significantly improved during the one-year follow-up.

CONCLUSIONS
Inhibition of allograft inflammatory activity and attenuation of the coronary endothelial dysfunction observed in cardiac transplant recipients during treatment with simvastatin may represent an important mechanism by which HMG-CoA reductase inhibitors protect against the development of cardiac allograft vasculopathy. (J Am Coll Cardiol 2001;38: 814–8) © 2001 by the American College of Cardiology

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Heart Transplant

PATIENTS AND METHODS
Patient population. This investigation was performed with approval by the Institutional Ethics Committee. Informed consent was obtained from all subjects. The study group consisted of 80 consecutive patients undergoing routine diagnostic coronary angiography one to three years after orthotopic heart transplantation. Patients were selected from a group of 108 consecutively studied transplant recipients (one to three years after transplantation) according to predefined exclusion criteria: acute rejection (International Society of Heart and Lung Transplantation grade ≥1b; n = 2) or infection episode (C-reactive protein [CRP]
Abbreviations and Acronyms

- ACh: acetylcholine
- CRP: C-reactive protein
- CFVR: coronary flow velocity reserve
- HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A
- IL: interleukin
- IVUS: intravascular ultrasound
- TNF: tumor necrosis factor
- ACE: angiotensin-converting enzyme
- CMV: cytomegalovirus
- HLA: human leukocyte antigen

≥1 mg/dl; n = 4) at the time of the study, serum creatinine >1.8 mg/dl (n = 9), glutamic-pyruvic-transaminase >120 mg/dl (n = 4) or fasting glucose concentration >140 mg/dl (n = 3). Moreover, patients with angiographically visible coronary artery disease were excluded (n = 6). The remaining 80 patients were in the fasting state, and all cardiovascular medications (angiotensin-converting enzyme inhibitors, calcium-antagonists, beta-blockers and aspirin) had been discontinued for at least 24 h. All patients studied were maintained on a tacrolimus and mycophenolate mofetil-based immunosuppression protocol. The 80 patients of this study were divided into three groups according to their history of hypercholesterolemia and their total serum cholesterol level at the time of the study. Escobar et al. (7) have demonstrated that total cholesterol level is an independent predictor of severity of cardiac allograft vasculopathy as detected by intravascular ultrasound (IVUS). A total cholesterol level of >240 mg/dl was used for the definition of hypercholesterolemia (5,8). Venous blood specimens were obtained after a 12-h fast. Group 1 consisted of 21 patients who had been previously (≥6 months) diagnosed with hypercholesterolemia and had reduced their total cholesterol levels to <240 mg/dl using 10 mg simvastatin. Group 2 consisted of 19 patients who had been previously (≥6 months) diagnosed with hypercholesterolemia and had failed to reduce their total cholesterol levels to <240 mg/dl despite 10 mg simvastatin treatment. Group 3 consisted of 40 patients without hypercholesterolemia and without simvastatin treatment. The number of patients at one, two and three years after transplant in each of the three study groups were not significantly different. Detailed patient characteristics are listed in Table 1. The prospective study groups consisted of 11 simvastatin-treated patients and 9 normocholesterolemic controls. Patients were studied before starting simvastatin (baseline) and after a follow-up period of 12 months.

Methods. First, a routine biopsy was taken via a transvenous approach to exclude acute rejection. A 6F multipurpose catheter was then placed in the coronary sinus, and a blood sample (10 ml) was withdrawn for determination of cytokine concentrations (interleukin [IL]-6, soluble interleukin [sIL]-2R, tumor necrosis factor [TNF]-α). Blood samples (10 ml) were withdrawn from the aortic root for determination of circulating cytokines. Measurements of coronary vasomotor response (quantitative coronary angiography and intracoronary Doppler flow measurement) and determination of intimal thickening (IVUS) have been described in detail elsewhere (9,10). In brief, following the diagnostic procedure including left ventriculography and coronary angiography, a Doppler Flow-wire (FloWire, Cardiometrics Inc., Mountain View, California) was placed in the proximal left anterior descending artery, permitting measurement of coronary blood flow velocities. A dosage of 5,000 IU of heparin was given intravenously. Blood flow velocity was recorded continuously during administration of the study agents. First, adenosine (80 and 160 μg/min over 5 min each; Adreka, Sanofi-Winthrop, Munich, Germany) was infused into the left coronary system to achieve maximal (endothelium-independent) coronary flow. Second, intracoronary acetylcholine ([ACh] 1 and 30 μg/min over 5 min each; Acetylcholine Dispera, Germering, Germany) was infused to investigate epicardial and microvascular endothelial vasomotor function (estimated final blood concentrations in the coronary bed of 10⁻⁷ and 3 × 10⁻⁸ mol/l). Third, intracoronary nifedipine (Adalat; Bayer AG, Leverkusen, Germany) was administered as a bolus

<table>
<thead>
<tr>
<th>Table 1. Patient Characteristics</th>
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<tbody>
<tr>
<td><strong>Group 1</strong> (Hypercholesterolemia Adequately Treated, n = 21)</td>
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<tr>
<td>Time after transplantation (months)</td>
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<tr>
<td>Recipient age (yr)</td>
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<tr>
<td>Donor age (yr)</td>
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<tr>
<td>Ischemic time (min)</td>
</tr>
<tr>
<td>Frequency of CMV mismatches</td>
</tr>
<tr>
<td>Number of HLA mismatches</td>
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<tr>
<td>Number of treated rejection episodes</td>
</tr>
<tr>
<td>Frequency of corticosteroids</td>
</tr>
<tr>
<td>Frequency of ACE-inhibitor treatment</td>
</tr>
<tr>
<td>Serum lipids</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
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</table>

*p < 0.01 vs. groups 1 and 3.

ACE = angiotensin-converting enzyme; CMV = cytomegalovirus; HLA = human leukocyte antigen.
Quantitative coronary angiography. Coronary angiography was performed to investigate epicardial arteries. In this study, no epicardial constriction >50% in response to ACh was observed. Therefore, diameter changes were not included in the calculation of CFVR. Coronary microvascular endothelial dysfunction has been defined as CFVR <2 in response to ACh (10).

Quantitative coronary angiography. Quantitative coronary angiography was performed to investigate epicardial vasomotor response using a computerized automatic-analysis system (HICOR, Siemens, Erlangen, Germany). Nonstenotic proximal and distal coronary arterial segments identified between easily visualized branch points were selected for analysis in the left anterior descending artery. Responses of the coronary segments to the different stimuli were expressed as percent change versus control value. The diameter change for each vessel was calculated on the basis of the average of the two segments. Intraobserver and interobserver variability showed high reproducibility (r of the average of the two segments. Intraobserver and diameter change for each vessel was calculated on the basis of the average of the two segments. Intraobserver and interobserver variability showed high reproducibility (r > 0.89, p < 0.001). Epicardial endothelial dysfunction has been defined as >10% diameter reduction in response to ACh compared with baseline (10).

IVUS. Immediately after Doppler flow measurement, IVUS was performed to detect atherosclerotic plaques not visible with angiography. The imaging system consisted of a 30-MHz ultrasound transducer enclosed within an acoustic housing on the tip of a 2.9F flexible, rapid-exchange catheter (CVIS Inc., Sunnyvale, California). The catheter was advanced to the distal left anterior descending or circumflex artery, carefully observing a lumen-IVUS catheter-diameter ratio of >1.5. During the subsequent standardized pullback maneuver, images were documented on SVHS videotape for further off-line analysis. The three sites with the most severe intimal proliferation were evaluated, and the averaged maximal intimal thickness was calculated (11). Maximal intimal thickness was measured as the greatest distance from the intimal leading edge to media-adventitia border. Measurements of lumen and vessel area were made of the three most severely stenosed sites and averaged. These analyses were performed by observers without knowledge of the results of the endothelial function or the lipid and cytokine levels.

Cytokine measurements. Measurement of cytokines using enzyme-linked immunosorbent assay was performed as described in detail elsewhere (9).

Statistical analysis. Continuous variables are presented as mean ± SD. When comparing three groups, one-way analysis of variance was followed by the Student-Newman-Keuls post hoc test for statistical significance. For comparisons within the same individuals over time (prospective study groups), the paired samples t test was used. Discrete variables are presented as percentages. The Pearson chi-square test was used to determine significant differences. Statistical significance was assumed if the null hypothesis could be rejected at the 0.05 probability level.

RESULTS

Coronary intimal thickening. Averaged maximal intimal thickness in groups 1 and 2 were similar and significantly (p < 0.01 and p < 0.05, respectively) larger than intimal thickness in group 3: 0.7 ± 0.3 mm (group 1), 0.6 ± 0.4 mm (group 2) and 0.4 ± 0.2 mm (group 3). Importantly, lumen areas in groups 1 and 3 were similar and significantly (p < 0.05) larger than lumen areas in group 2: 7.5 ± 0.6 mm² (group 1), 6.3 ± 0.5 mm² (group 2) and 7.9 ± 0.3 mm² (group 3).

Coronary vasomotor response. Results are referred to the highest dosage administered of each drug. The values of heart rate and mean blood pressure after drug administration were not significantly altered compared to baseline (data not shown). Epicardial luminal diameter constriction in response to ACh was significantly more pronounced in group 2 compared with group 1 and group 3 patients (p < 0.05; Fig. 1). Vasoconstrictor response to ACh in group 3 tended to be increased compared with group 1 (p = 0.07). Endothelium-independent responses to adenosine and nifedipine were similar in all groups (data not shown). Moreover, endothelium-dependent flow velocity increase was similar in group 1 and group 3; the increases were significantly greater than those in group 2 (p < 0.05; Fig. 2). Endothelium-independent microvascular responses were comparable between the groups (data not shown).

Cytokine concentrations. Plasma cytokine concentrations in the coronary sinus and aortic root are depicted in Table 2. Cardiac IL-6 and TNF-α release was significantly lower in group 1 than it was in groups 2 and 3 (for respective values in group 1, 2 and 3: IL-6: −2 ± 2 pg/ml, 3 ± 4 pg/ml and 6 ± 6 pg/ml; all p < 0.05; TNF-α: −1 ± 4 ng/ml, 7 ± 5 ng/ml and 6 ± 4 ng/ml; all p < 0.01). Interleukin-2R concentrations were similar in all groups.
Prospective study. Characteristics of plaque and coronary hemodynamics, as well as lipid and cytokine concentrations before and after simvastatin treatment are shown in Table 3. Coronary epicardial and microvascular endothelial dysfunction significantly improved after one year of simvastatin treatment and was associated with a decrease in cardiac IL-6 and TNF-α release and an increase in coronary lumen area (Table 3). In contrast, functional and morphological coronary features as well as cytokine concentrations were unchanged in heart transplant recipients without simvastatin treatment.

DISCUSSION

This study demonstrates that simvastatin treatment in transplant patients is associated with a better epicardial and microvascular endothelial function dependent on the amount of cholesterol lowering. These changes were associated with decreased cardiac cytokine release demonstrating simvastatin-mediated anti-inflammatory activity.

Statins and inflammation. Our findings support the notion of HMG-CoA reductase inhibitors as immunomodulating agents with downregulation of inflammatory responses (12). Atherogenic lipoproteins, in particular low-density lipoprotein, are potent proinflammatory stimuli eliciting a broad spectrum of cellular responses. Effectively lowering the concentrations of these lipoproteins may, therefore, normalize systemic inflammation markers. However, the cytokine concentrations in the adequately treated group was, in fact, lower than the concentrations in normo-cholesterolemic subjects, pointing to an at least partial lipid-independent mechanism. Importantly, in the Cholesterol And Recurrent Events trial, mean CRP levels decreased nearly 40% during a five-year period among those patients allocated to pravastatin versus placebo, an effect not related to pravastatin-induced changes in low-density lipoprotein cholesterol (13).

Statins, endothelial function and vascular remodeling. Moreover, our results are consistent with previous studies in nontransplant humans that demonstrated improvement in peripheral and coronary endothelial function after treatment with inhibitors of the HMG-CoA reductase (3–5). Intimal thickening as an early marker of morphological allograft vasculopathy was significantly elevated in patients with hypercholesterolemia not adequately controlled. This is consistent with two prospective clinical trials demonstrating significantly reduced increases of the intimal thickness after cardiac transplantation in patients treated with HMG-CoA reductase inhibitors (1,2). They also reported on a reduced prevalence of acute rejection episodes and better survival. In contrast, in this study intimal thickness was increased in patients with hypercholesterolemia adequately controlled compared with patients without a history of hypercholesterolemia. This may result from a relatively short treatment period (8 ± 5 months) not sufficient enough to have impact on atherosclerotic plaque regression. However, coronary lumen area in adequately controlled hypercholesterolemic patients was significantly increased compared with uncontrolled patients demonstrating adaptive coronary remodeling. Interestingly, endothelial function may modulate the process of vascular remodeling (14). In fact, in the present prospective study, simvastatin treatment reduced coronary artery constriction and increased coronary blood flow responses during intracoronary acetylcholine infusion, reflecting improved endothelial–dependent action in conduit coronary arteries and coronary microvessels, respectively. Additionally, simvastatin treatment was associated with an improvement in coronary lumen area that resulted not from

Table 2. Cytokines

<table>
<thead>
<tr>
<th>Group</th>
<th>Group 1 (Hypercholesterolemia Adequately Treated, n = 21)</th>
<th>Group 2 (Hypercholesterolemia Inadequately Controlled, n = 19)</th>
<th>Group 3 (Without Hypercholesterolemia, n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>12 ± 10</td>
<td>18 ± 7</td>
<td>15 ± 12</td>
</tr>
<tr>
<td>IL-6</td>
<td>15 ± 6</td>
<td>16 ± 10</td>
<td>11 ± 9</td>
</tr>
<tr>
<td>IL-6</td>
<td>−2 ± 2†</td>
<td>3 ± 4</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>TNF-α</td>
<td>17 ± 9</td>
<td>21 ± 9</td>
<td>22 ± 7</td>
</tr>
<tr>
<td>TNF-α</td>
<td>17 ± 10</td>
<td>15 ± 12</td>
<td>17 ± 7</td>
</tr>
<tr>
<td>TNF-α</td>
<td>−1 ± 4†</td>
<td>7 ± 5</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>IL-2R</td>
<td>0.8 ± 0.6</td>
<td>0.7 ± 0.5</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>IL-2R</td>
<td>0.9 ± 0.4</td>
<td>0.7 ± 0.7</td>
<td>0.6 ± 0.9</td>
</tr>
<tr>
<td>IL-2R</td>
<td>−0.03 ± 0.05</td>
<td>0.03 ± 0.1</td>
<td>0.06 ± 0.1</td>
</tr>
</tbody>
</table>

*p < 0.01 vs. groups 2 and 3; †p < 0.05 vs. groups 2 and 3.
IL = interleukin; TNF = tumor necrosis factor; transcardiac gradient = coronary sinus minus aortic concentrations.
a decrease in intimal area but from an increase in vessel area, reflecting vascular remodeling.

**Study limitations.** The limitations of intracoronary ultrasound imaging and Doppler flow velocity measurement have been described in detail elsewhere (15,16). Focusing of the three worst sites of each vessel may underestimate the severity of diffuse intimal thickening. Therefore, further studies with plaque volumetry in several predefined segments may improve the comparability of the measurements. Moreover, we cannot rule out that functional and morphological coronary features were different between the three groups before statin treatment. Indeed, this study is mainly a cross-sectional study, and findings may warrant confirmation through a larger prospective study.

In summary, the better epicardial and microvascular endothelial vasomotor function in heart transplant recipients treated with simvastatin may result, at least in part, from an attenuated inflammatory response in the cardiac allograft. Improvement in endothelial dysfunction was associated with adaptive coronary remodeling.

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**REFERENCES**


