Coronary Endothelial Dysfunction After Heart Transplantation Predicts Allograft Vasculopathy and Cardiac Death

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Background—Coronary endothelial dysfunction may be an early marker for cardiac allograft vasculopathy (CAV) in orthotopic heart transplant recipients. Using serial studies with intravascular ultrasound and Doppler flow-wire measurements, we have previously demonstrated that annual decrements in coronary endothelial function are associated with progressive intimal thickening. The present study tested whether endothelial dysfunction predicts subsequent clinical events, including cardiac death and CAV development.

Methods and Results—Seventy-three patients were studied yearly beginning at transplantation until a prespecified end point was reached. End points were angiographic evidence of CAV (>50% stenosis) or cardiac death (graft failure or sudden death). At each study, coronary endothelial function was measured with intracoronary infusions of adenosine (32-μg bolus), acetylcholine (54 μg over 2 minutes), and nitroglycerin (200 μg) into the left anterior descending coronary artery; intravascular ultrasound images and Doppler velocities were recorded simultaneously. Of the 73 patients studied, 14 reached an end point during the study (6 CAV and 8 deaths, including 4 with known CAV, 1 graft failure, and 3 sudden). On the last study performed, the group with an end point had decreased epicardial (constriction of 11.1 ± 2.9% versus dilation of 1.7 ± 2.2%, \( P = 0.01 \)) and microvascular (flow increase of 75 ± 20% versus 149 ± 16%, \( P = 0.03 \)) endothelium-dependent responses to acetylcholine compared with the patients who did not reach an end point. Responses to adenosine and nitroglycerin did not differ significantly.

Conclusions—Endothelial dysfunction, as detected by abnormal responses to acetylcholine, preceded the development of clinical end points. These data implicate endothelial dysfunction in the development of clinically significant vasculopathy and suggest that serial studies of endothelial function have clinical utility. (Circulation. 2001;104:3091-3096.)

Key Words: acetylcholine ■ endothelium ■ transplantation ■ atherosclerosis ■ vasculature

Cardiac allograft vasculopathy (CAV) is a major cause of long-term morbidity and mortality after successful heart transplantation and represents the leading cause of mortality in transplant recipients who survive more than 1 year.\(^1\) CAV is progressive and tends to be diffuse, involving both large and small coronary vessels.\(^2\) The progression of CAV may be rapid and without associated symptoms of angina in the denervated heart. Consequently, patients may present with unexpected sudden death, congestive heart failure, or arrhythmias.\(^3\) Noninvasive assessment has lacked adequate sensitivity and specificity to predict CAV and clinical outcomes.\(^4\) For this reason, annual coronary angiography is performed for diagnostic and surveillance purposes. Angiography, however, is less sensitive than intravascular ultrasound (IVUS) for detection of the earliest stages of CAV. In the most recent multi-institutional study,\(^5\) angiographically visible CAV was present in 42% of patients (27% mild, 8% moderate, and 7% severe). With the use of IVUS, intimal thickening is detected in up to 75% of patients at 1 year.\(^6\) However, no clear relationship between intimal thickening and subsequent CAV detected by angiography has been demonstrated.

This study sought to determine whether coronary endothelial dysfunction precedes angiographically and clinically evident CAV and to clarify the degree to which the presence of endothelial dysfunction predicts the subsequent development of significant CAV and clinical events. Over a 7-year period, serial evaluations of endothelial function were performed on heart transplant recipients at annual angiography, starting with the initial study just after transplantation, by use of simultaneous IVUS and Doppler flow studies to assess responses to intracoronary infusion of acetylcholine, adenosine, and nitroglycerin. With this methodology, this technique
has been shown to distinguish between epicardial and microvascular responses and between agonist-mediated and flow-mediated endothelium-dependent responses. In longitudinal studies, we have shown that endothelial dysfunction predicts future progression of coronary intimal thickening in heart transplant patients, with microvascular responses to acetylcholine being stronger predictors than large-vessel responses. The present study reports the relationship between coronary endothelial dysfunction and the clinical end points of angiographically significant coronary allograft vasculopathy, ischemic events, and death in heart transplant recipients.

Methods

Patient Population

The study cohort consisted of heart transplant recipients undergoing posttransplant angiography at Rush Presbyterian-St Luke’s Medical Center. Patients <18 years of age, patients with known angiographic evidence of coronary artery stenosis exceeding 50% of luminal diameter, and patients with evidence of acute rejection by endomyocardial biopsy were excluded. Informed consent was obtained from all patients according to the guidelines of the Rush Presbyterian-St Luke’s Medical Center Human Investigation Committee. Patients were studied in the fasting state, and long-acting vasoactive medications were discontinued at least 18 hours before the study; short-acting vasoactive medications were withheld on the day of the study.

Study Protocol

After diagnostic coronary angiography, an intervention guiding catheter was selectively positioned in the left coronary artery, and a 10,000-U heparin bolus was administered intravenously. Serum activated clotting time was checked and maintained for >300 seconds throughout. A 0.014-in Doppler flow wire (Flower; Cardiometrics) was inserted in the left anterior descending coronary artery (LAD). A 3.5F or 3.2F 30-MHz IVUS catheter (Sonicath; Boston Scientific Co, and CVIS) was passed over the flow wire and positioned in the LAD immediately distal to the first septal perforating branch. The Doppler flow wire was positioned several centimeters distal to the terminal portion of the IVUS catheter to avoid artifacts caused by the catheter wake. A sample recording is shown in Figure 1. The position of the IVUS catheter and flow wire was documented by angiography.

Intracoronary Infusions

Serial intracoronary infusions were performed according to an established protocol. First, adenosine (Adenocard; Fujisawa USA Inc) was administered as a 16-μg intracoronary bolus via the guide catheter. Intravascular images and coronary velocity measurements were recorded concurrently with adenosine injection; recording continued until vessel diameter and coronary flow velocity returned to baseline. After this, a 32-μg adenosine bolus was given via the guide catheter, with recording of coronary area by IVUS and of velocity by Doppler wire. On return to the basal state, 5.4 μg of acetylcholine chloride (Miochol, Baxter) in 10 mL of 5% dextrose was infused over 2 minutes via the guide catheter with a syringe pump (Harvard Apparatus; estimated final intracoronary concentration 0.1 μmol/L, assuming left main coronary flow of 150 mL/min). Because of the dead space in the tubing (5 mL), acetylcholine reaches the coronary circulation after a 60-second interval; response times were measured after this interval. IVUS and coronary flow velocity data were acquired throughout the infusion and for 2 minutes afterward. After return to baseline, 54 μg of acetylcholine was infused over 2 minutes (estimated final intracoronary acetylcholine concentration of 1 μmol/L), again with continuous acquisition of data. Finally, after return to the basal state, an intracoronary bolus of 200 μg of nitroglycerin was given via the guide, with continuous recording of IVUS lumen area and Doppler velocity.

During each infusion, heart rate and ECG were monitored continuously. Arterial pressure was monitored continuously via the guiding catheter after bolus infusions and periodically during the continuous infusions. After the intracoronary infusions, the IVUS catheter and Doppler flow wire were removed, and coronary angiography was repeated to confirm vessel patency and absence of vasospasm. Both adenosine and acetylcholine have negative chronotropic effects and can cause heart block and even asystole. Transient bradycardia was noted in 7 patients after intracoronary bolus administration of adenosine, with transitory heart block in 3 of these patients. This resolved within seconds and was not associated with hemodynamic compromise. Asystole did not occur in the present study. Bradycardia was observed in 21 patients with intracoronary acetylcholine infusion, with transient heart block in 18. When this occurred, the infusion was stopped; the bradycardia always resolved within seconds and was not associated with hemodynamic compromise.

Analysis

IVUS images were recorded on Super VHS tape and then digitized into a Macintosh computer for analysis. Intimal thickness and intimal index (percentage of vessel area taken up by intima) were determined from IVUS pullbacks with NIH-Image by two observers blinded to the Doppler flow results. Average intimal index was calculated by morphometric analysis of 10 equally spaced segments from IVUS pullback.

Doppler flow velocity measurements were recorded on tape for subsequent analysis. Velocity-time integrals were obtained from Doppler flow envelopes by use of the software on a Hewlett-Packard 1500 echocardiography machine. Absolute coronary flow at each time point was calculated by multiplying Doppler-derived mean flow velocity by lumen area measured by IVUS. Mean flow velocity was derived from the Doppler wire measurements in accordance with a previously validated formula by multiplying the time average of spectral peak velocity by 0.5.

Results presented here represent comparison of the last study before development of an end point (angiographic vasculopathy was an exclusion criterion and an end point) with the last study performed in patients without a clinical end point. The mean time from the last study to development of an end point was 9±3 months.

Statistics

For each drug infusion, peak coronary area, peak velocity, and peak flow were determined; the maximum responses at the highest drug concentration given are expressed as a percentage of baseline and presented as mean±1 SD. Responses between groups were compared by Student t tests. Probability values less than 0.05 were deemed statistically significant.

Results

Patient Characteristics

The mean age of the 73 study patients was 49±12 years (range 18 to 64 years), and mean donor age was 28±13 years.
(range 11 to 55 years). Sixty-four patients were male. The pretransplant diagnosis was coronary artery disease in 35 (48%) and nonischemic cardiomyopathy in 38 (52%). All patients were treated with triple immunosuppressive therapy (prednisone, cyclosporine, and azathioprine or mycophenolate mofetil) and pravastatin. Calcium channel antagonists were not routinely prescribed for prevention of allograft vasculopathy, but 14 patients were receiving calcium channel blockers for treatment of hypertension.

**Outcomes**

The prespecified prospectively defined end points were angiographically significant CAV with reduction of lumen diameter by at least 50%, ischemic events, and death more than 1 year after heart transplantation. Over the course of the study, 14 patients reached one of these end points; 6 developed significant coronary allograft vasculopathy, and 8 died. Of the 8 deaths, 4 patients had known allograft vasculopathy, 1 died of graft failure, and 3 died suddenly.

The demographics of patients who developed an end point compared with those who did not are summarized in Table 1. Neither age, blood pressure, time from transplantation, or number of studies performed differed between the 2 groups. The latter 2 points are important, because they exclude time as a confounding factor in the assessment of endothelial function.

**Endothelial Function**

Endothelium-independent epicardial vasodilatory responses to nitroglycerin did not differ between the 2 groups. In the patients with end points had vasodilation in response to nitroglycerin from a mean lumen area of 14.9±2.4 to 16.2±2.7 mm², a mean increase of 10.4±7.1%, and those without end points dilated from a mean area of 14.6±2.2 to 16.5±2.3 mm², an increase of 14.6±5.2% (P=0.20; Figure 2).

Endothelium-independent microvascular flow responses to adenosine also did not differ between the 2 groups. In the patients with end points, mean lumen area was 14.6±2.2 mm², mean flow velocity was 22.2±6.3 cm/s, and calculated LAD flow was 1.54±0.49 mL/s. After intracoronary adenosine, lumen area increased to 15.8±2.5 mm², flow velocity increased to 56.6±10.6 cm/s, and calculated flow increased to 4.64±0.82 mL/s, a mean increase of 203±75%.

In the patients without end points, mean lumen area increased from 14.7±2.1 to 15.9±2.3 mm², mean flow velocity from 24.1±3.7 to 60.1±8.9 cm/s, and calculated LAD flow from 1.55±0.33 to 4.21±0.87 mL/s, an increase of 175±78% (P=0.42 compared with group with end points; Figure 3).

**Table 1. Demographics in Patients With and Without a Clinical End Point**

<table>
<thead>
<tr>
<th></th>
<th>Patients Who Reached an End Point (n=14)</th>
<th>Patients Not Reaching an End Point (n=59)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>48±13</td>
<td>49±10</td>
<td>0.85</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>141±18</td>
<td>140±18</td>
<td>0.90</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>89±11</td>
<td>89±13</td>
<td>0.89</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>106±14</td>
<td>106±12</td>
<td>0.88</td>
</tr>
<tr>
<td>Mean time from transplantation, mo</td>
<td>30±6 (median 33)</td>
<td>32±3 (median 35)</td>
<td>0.83</td>
</tr>
<tr>
<td>Number of studies</td>
<td>2.7±1.3</td>
<td>2.8±1.4</td>
<td>0.91</td>
</tr>
</tbody>
</table>

**Figure 2.** Change in epicardial coronary artery area in response to nitroglycerin in transplant patients who did (n=14) or did not (n=59) reach a clinical end point. Results are expressed as percent change from baseline. No significant difference was found.

**Figure 3.** Change in microvascular coronary artery flow in response to adenosine in transplant patients who did (n=14) or did not (n=59) reach a clinical end point. Results are expressed as percent increase from baseline. No significant difference was found.
Table 2. Sensitivity, Specificity, and Predictive Value of Abnormal Endothelial Function for Clinical End Points

<table>
<thead>
<tr>
<th>Response</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PVP</th>
<th>PVN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal epicardial response</td>
<td>86%</td>
<td>64%</td>
<td>36%</td>
<td>94%</td>
</tr>
<tr>
<td>Abnormal microvascular response</td>
<td>79%</td>
<td>69%</td>
<td>38%</td>
<td>93%</td>
</tr>
<tr>
<td>Any abnormal endothelial response</td>
<td>100%</td>
<td>54%</td>
<td>31%</td>
<td>100%</td>
</tr>
</tbody>
</table>

PVP indicates predictive value of a positive test; PVN, predictive value of a negative test.

The patients without end points had paradoxical vasoconstriction (>5%). In the patients with end points, acetylcholine caused a mean epicardial constriction of 11.1±2.9%, with paradoxical vasoconstriction in 12 of 14. This response differed significantly from those without end points (P=0.01; Figure 4). The increase in flow after acetylcholine administration, an index of microvascular endothelial function, was 149±16% in patients without end points and 75±20% in patients with end points, also a significant difference (P=0.03; Figure 5).

When serial responses were evaluated, patients with end points had a more rapid decrease in endothelial function than patients without end points. The mean annual decrement in area response to acetylcholine in patients with end points was 3.07±1.83%, whereas patients without end points had a smaller annual decrement in area response of 1.02±1.07% (P<0.05). Patients with end points had a mean annual decrement in flow response to acetylcholine of 14.0±10.8%, and the annual decrease in patients without end points was 9.9±11.6% (P=0.27).

The sensitivity and specificity of an abnormal endothelial response for development of a clinical end point are shown in Table 2. Abnormal epicardial responses were defined as dilation to acetylcholine ≥5% and abnormal microvascular responses as flow increase <100% (flow reserve <2.0). The sensitivity and specificity of an abnormal epicardial response were 86% and 64%. For an abnormal microvascular response, sensitivity was 79% and specificity was 69%. The sensitivity of any abnormal endothelial response was 100%, with specificity of 54%.

**Discussion**

Although most of the evidence indicates that CAV is a form of chronic allograft rejection, it is clear that the immunologic mechanisms that lead to intimal proliferation in transplant recipients operate in a milieu of nonimmunologic risk factors, including hyperlipidemia, hypertension, viral infections, and donor predisposition. In one longitudinal study, independent predictors of severe allograft vasculopathy included first-year mean biopsy score, donor age, and hyperlipidemia.

The vascular endothelium, situated at the interface between the circulating immune cells and the vessel wall, is an early target of the immune system after transplantation. Chronic allograft endothelial damage leads to intimal proliferation, macrophage migration, and eventual smooth muscle hyperplasia. It is also well recognized that hyperlipidemia, hypertension, hyperglycemia, and other risk factors can also cause endothelial dysfunction. Regardless of the specific initiator of endothelial injury, the cascade of events that follows involves inflammatory responses. Thus, immune- and inflammatory-mediated endothelial cell injury can alter endothelial function and contribute to the development of CAV.

The use of simultaneous IVUS and Doppler wire instrumentation in the present study allowed the evaluation of endothelial function in both the epicardial vessels and microvasculature of heart transplant patients. The patients in the present study represent the largest reported study with serial assessment of endothelial function. The main finding was that coronary endothelial dysfunction predicted hard cardiovascular end points after heart transplantation.

Our group has previously used this technique to show that coronary endothelial dysfunction can predict future progression of coronary intimal thickening in heart transplant patients. Although IVUS can detect vessel wall abnormalities in angiographically normal coronary arteries, the significance of intimal thickening is not entirely clear. It seems likely that
all patients who ultimately develop severe intimal thickening will demonstrate progressive degrees of intimal thickening on serial studies, but not all patients progress, and progression occurs at different rates. In one series of 70 transplant patients imaged with IVUS a mean of 3.1 years after transplantation and 1 year later, the amount of initial thickening did not predict progression of intimal proliferation or the progression to angiographically detectable disease. Other groups have also failed to find a relationship between intimal thickening and progression to angiographic CAV. It is hypothesized that what may be most important in early transplant atherosclerosis is not the absolute degree of intimal thickening but rather the rate of progression of disease. Our previous findings in this cohort of patients support the notion that endothelial function may serve as a marker for the rate of progression of transplant atherosclerosis. The present study has now extended these findings by demonstrating that endothelial dysfunction is predictive of important clinical endpoints.

Normal endothelial function plays a central role in vascular homeostasis, including regulation of vascular tone, inhibition of thrombus formation, inhibition of leukocyte adhesion, and regulation of vascular smooth muscle proliferation. Dysfunction of the coronary vascular endothelium, as assessed by decreased endothelium-dependent vasodilation, is an early and characteristic feature of both native and allograft coronary artery disease. Decreased local availability of nitric oxide (NO) could contribute to both the initiation and progression of atherosclerosis. NO modulates both platelet–vessel wall and leukocyte–vessel wall interactions; diminished NO bioavailability could thus allow increased platelet adhesion and the potential for increased local concentrations of platelet-derived growth factor, as well as increased adhesion of monocytes and other inflammatory cells. Such adhesion in the presence of decreased NO could increase local oxidant stress, potentially oxidizing lipoproteins and increasing expression of oxidant-sensitive inflammatory genes. NO also plays an important role in regulating smooth muscle proliferation, and decreased NO activity could potentiate neointimal proliferation. In addition, because plaque instability is related to the degree of inflammation, endothelial dysfunction could increase the potential for acute coronary events. These mechanistic considerations lend plausibility to the notion that endothelial dysfunction could exacerbate the progression of CAV in transplant recipients.

Abnormal endothelial function was quite sensitive for the subsequent development of clinical end points but predictably was less specific. This would suggest that a single measurement of endothelial function would be most useful if normal, because the negative predictive value is high. Our previous studies have suggested that at least with respect to progression of coronary intimal thickening, changes in endothelial function from one study to another are better predictors than single determinations. This should be expected, because the underlying pathophysiological processes are dynamic rather than linear. Whether the change in endothelial function on serial studies would have improved predictive value will require further investigation.

Study Limitations
Epicardial area responses and microvascular flow responses were measured simultaneously, but these measurements were not obtained at the same point in the coronary tree because of IVUS catheter design and the need to avoid flow artifacts from the catheter wake. Continuous measurement of both parameters allowed for calculation of the maximal response, and we have shown that maximal area and flow responses do not occur at the same time. In addition, epicardial responses were measured at only one point in the proximal portion of the coronary tree. Although CAV is a diffuse process, there is good evidence for segmental heterogeneity in endothelium-dependent responses in different coronary arteries and at different sites in the same coronary artery.

Conclusions
Simultaneous IVUS and Doppler measurements were performed for serial assessment of both epicardial and microvascular endothelial function in heart transplant patients. This study is the first to show that coronary endothelial dysfunction predicts significant cardiovascular end points after heart transplantation. These data implicate endothelial dysfunction in the development of clinically significant CAV. Coronary endothelial vasodilator dysfunction in this population may represent an index that integrates the overall stress imposed by chronic rejection and by traditional coronary risk factors. Serial studies of endothelial function can play an important role in predicting clinical outcomes after cardiac transplantation.

Acknowledgments
This work was supported in part by a Grant-in-Aid from the Metropolitan Chicago American Heart Association and in part by a SmithKline Beecham Junior Faculty Development Award to Dr Hollenberg.
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_Circulation_. 2001;104:3091-3096
doi: 10.1161/hc5001.100796

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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