Brief Rapid Communication

Endothelial Dysfunction Early After Heart Transplantation
Assessment With Intravascular Ultrasound and Doppler

Roger M. Mills Jr., MD; Jeanette M. Billett, MD; and Wilmer W. Nichols, PhD

Background. Allograft vasculopathy after heart transplantation is thought to represent a response to endothelial injury in the graft vessels. To assess endothelial function before the onset of anatomic disease, coronary vasomotor responses to adenosine, acetylcholine, and nitroglycerin were evaluated in transplant recipients by intravascular ultrasound imaging and Doppler flow studies.

Methods and Results. Nine patients were studied 1 year after heart transplantation. Acetylcholine provoked significant vasoconstriction to 82% of maximal coronary diameter but was associated with an increase in mean coronary blood flow from 63.1 to 204 ml/min. Coronary blood flow increased fivefold in response to adenosine, a normal response.

Conclusions. The vasomotor response to acetylcholine at 1 year after heart transplantation is consistent with endothelial dysfunction in the epicardial conduit vessels. Microvascular function as judged by coronary flow reserve appears to be normal. (Circulation 1992;86:1171–1174)

KEY WORDS • adenosine • acetylcholine • nitroglycerin • endothelium • coronary flow reserve

Increasing clinical experience with heart transplantation has led to first-year survival rates of 85% or more; long-term success is limited, however, by the development of cardiac allograft vasculopathy, a unique form of coronary artery disease in the graft.1 This process now accounts for the majority of late postoperative deaths in heart transplant recipients.

The “response-to-injury” hypothesis regarding the pathogenesis of atherosclerotic disease2,3,4 is also believed to apply to allograft vasculopathy. This hypothesis states that endothelial cell injury initiates smooth-muscle cell migration and proliferation in the transplanted coronary arteries, leading to progressive concentric narrowing. To test the proposition that endothelial injury occurs early after transplantation, we studied coronary vasomotor responses to infusion of nitroglycerin, adenosine, and acetylcholine (ACh) using intravascular ultrasound to directly observe changes in lumen diameter in nine patients undergoing coronary angiography 1 year after heart transplantation. Four of the nine patients also had intracoronary Doppler flow velocity measurements to assess microvascular function with the same agents.

Methods

The Institutional Review Board of Shands Hospital at the University of Florida approved this protocol. Patients participated with fully informed consent.

All patients received heparin (10,000 units i.v.) immediately after placement of sheaths in the femoral artery and vein. In those patients who had not had a permanent epicardial VVI pulse generator placed at the time of surgery as a part of a separate investigational protocol, a standby bipolar pacing lead was placed in the inferior vena cava.

A 5F, 30-MHz intracoronary ultrasound catheter (Cardiovascular Imaging Systems, Inc., Sunnyvale, Calif.) was positioned in the left anterior descending coronary artery (LAD) just proximal to the origin of the first large diagonal branch, using a minimum of intravascular contrast and standard interventional techniques, with a 9F guiding catheter and 0.014-in. guide wire. After satisfactory initial images were obtained, the study agents were injected into the left coronary system through the guiding catheter; the agents were given as a bolus within 10 seconds followed by a saline flush. All patients first received 2 mg adenosine followed by 60 μg ACh. After each drug injection, observation was continued until the vessel returned to its initial diameter. The five patients who did not have Doppler studies then received 150 μg nitroglycerin. In the four patients who had additional Doppler studies, after completion of the ultrasound imaging with adenosine and ACh, the ultrasound catheter was exchanged over the guide wire for a 20-MHz, 3F Doppler flow catheter (DC-101, Millar Instruments, Inc., Houston, Tex.). Adenosine 2 mg, ACh 60 μg, and nitroglycerin 150 μg were administered for flow studies, allowing flow to return to baseline after each agent. On completion of the study protocol, a diagnostic cine angiogram was filmed.

Real-time ultrasound imaging was recorded continuously throughout the protocol. Three to six diastolic frames showing maximal vasomotor responses after...
infusion of each agent were selected for analysis. The outline of the lumen at the black/white interface was traced on a video screen by use of a “joystick” on a standard echo-quantitation computer (Bruce Franklin, Inc., Woodenville, Wash.). The measurements were calibrated in tenths of a millimeter, and areas (A) were calculated in square millimeters. Coronary diameter (D) for each state was calculated from the average of three to six luminal areas as \( D = 2(A/\pi)^{1/2} \).

For patients undergoing Doppler flow studies, the Doppler catheter was positioned as nearly as possible in the same site as the ultrasound probe. The Doppler catheter was connected to a bidirectional 20-MHz velocimeter (Millar Instruments, Inc.) from which mean and phasic velocity signals were obtained and recorded on a photographic multichannel oscillographic recorder (VR-12, Electronics for Medicine, Pleasantville, N.Y.). Coronary flow velocity (in centimeters per minute) signals were calibrated in terms of volume flow (in milliliters per minute) using intraluminal cross-sectional area, obtained as described above. Coronary flow reserve (CFR) was calculated using volume blood flow as \( \text{CFR} = F_p/F_b \), where \( F_p \) is peak flow and \( F_b \) is baseline flow. Intravascular ultrasound images and the coronary cine angiography were carefully reviewed for any evidence of anatomic coronary disease.

**Results**

Table 1 shows the patient characteristics and mean LAD diameters for all patients. None of the patients had angiographic or intravascular ultrasound evidence of coronary vasculopathy. We found no significant difference in the coronary vasomotor responses to adenosine, ACh, and nitroglycerin, comparing by gender or current rejection status. Comparison of the six patients who had experienced either cytomegalovirus (CMV) infection or antirejection therapy with intravenous corticosteroids during the first year versus the three who had neither CMV infection nor significant rejection was not statistically significant. Those patients who had experienced either CMV infection or rejection, however, showed a strong trend toward increased vasoconstriction with ACh.

The mean LAD diameters in the nitroglycerin, initial, and ACh conditions expressed as a percentage of the adenosine diameter were 97.7±7.5%, 88.8±7.0%, and 83.8±8.8%, respectively. Repeated-measures ANOVA followed by Duncan’s multiple comparison procedure demonstrates that the nitroglycerin, initial, and ACh states are all significantly different (\( p<0.0001 \)).

The Doppler flow measurements with adenosine and ACh are shown in Table 2, and Figure 1 shows a typical Doppler flow study after ACh administration. All four patients showed increases in flow with both ACh and adenosine. By Duncan’s multiple comparison procedure, the mean adenosine and ACh flows are significantly higher than initial flow, and the coronary flow in response to adenosine is significantly higher than that in response to ACh (\( p<0.05 \)). Maximal responses occurred approximately 25–35 seconds after bolus adenosine, 20–30 seconds after ACh, and 15–25 seconds after nitroglycerin. The maximal vasoconstrictor response to ACh occurred during increased flow, with no evidence of flow-mediated dilation.

**Discussion**

These data show significant epicardial coronary artery vasoconstriction in response to ACh, consistent with endothelial dysfunction, in a series of nine patients studied prospectively 1 year after heart transplantation.

**Table 1.** Patient Characteristics and Mean Diameter of the Proximal Left Anterior Descending Coronary Artery Determined by Intravascular Ultrasound

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Rejection on study biopsy</th>
<th>CMV infection after transplantation</th>
<th>Prior rejection requiring intravenous steroids</th>
<th>LAD mean diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>45</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3.90</td>
</tr>
<tr>
<td>2</td>
<td>65</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>3.74</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>3.65</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.39</td>
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<tr>
<td>5</td>
<td>47</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>3.74</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>5.98</td>
</tr>
<tr>
<td>7</td>
<td>53</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>4.15</td>
</tr>
<tr>
<td>8</td>
<td>54</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.74</td>
</tr>
<tr>
<td>9</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.38</td>
</tr>
<tr>
<td>Mean</td>
<td>52.7</td>
<td></td>
<td></td>
<td></td>
<td>4.24</td>
</tr>
<tr>
<td>SD</td>
<td>8.1</td>
<td></td>
<td></td>
<td></td>
<td>0.76</td>
</tr>
</tbody>
</table>

CMV, cytomegalovirus; LAD, left anterior descending coronary artery; +, positive; 0, negative.

**Table 2.** Left Anterior Descending Coronary Artery Flow in Response to Adenosine and Acetylcholine and Coronary Flow Reserve with Adenosine

<table>
<thead>
<tr>
<th>Patient</th>
<th>LAD flow (ml/min)</th>
<th>CFR</th>
<th>Adenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>172</td>
<td>4.79</td>
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<tr>
<td>6</td>
<td>96</td>
<td>315</td>
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<tr>
<td>9</td>
<td>45</td>
<td>163</td>
<td>4.00</td>
</tr>
<tr>
<td>Mean</td>
<td>63*</td>
<td>204*</td>
<td>5.06</td>
</tr>
<tr>
<td>SD</td>
<td>26.6</td>
<td>74.1</td>
<td>0.98</td>
</tr>
</tbody>
</table>

LAD, left anterior descending coronary artery; CFR, coronary flow reserve.

*p<0.05.*
Subselective coronary blood flow increased significantly in response to ACh despite constriction of the epicardial vessel, suggesting that microvascular vasodilation remained intact. CFR as assessed with adenosine was normal. None of these patients had evidence of anatomic cardiac allograft vasculopathy by intravascular ultrasound or selective coronary angiography.

The coronary vasodilator response to non-endothelium-dependent agents remains intact after cardiac transplantation, as shown in both quantitative coronary angiographic and intravascular ultrasound studies. Variable responses to endothelium-dependent agents, including both ACh and substance P, have been reported. These observations have been complicated by 1) the need for contrast injection and quantitative coronary angiography, 2) variations in time from transplantation to time of study, 3) variable information concerning the patients' past and present rejection status and CMV status, 4) uncertainty concerning the appropriate baseline for quantitative observations, and 5) variations in dose and dose–response curve.

With intravascular ultrasound imaging, the vasodilator effect of contrast and the technical difficulties of quantitative coronary angiography are eliminated. The ability to continuously monitor the arterial response to vasoactive agents with intravascular ultrasound allows assessment of the maximal response to agents with brief pharmacological duration of action and eliminates the need for infusion. Intravascular ultrasound measurements have been shown to be reproducible and accurate in vitro and in vivo. The problem of an intense chronic vasoconstrictor state after cardiac transplantation and the possibility that this could alter apparent vasomotor responses led us to use the maximum coronary diameter observed after intracoronary adenosine as a standard and to express the diameters measured in other conditions as a percentage of the maximally vasodilated state. This approach provides a stable, reproducible anatomic basis for comparison, as advocated by other investigators.

In five patients with complete data sets, we found no significant difference between adenosine and nitroglycerin responses, which probably represent the true maximal coronary diameter. In contrast, when initial, nitroglycerin, and ACh diameters are expressed as a percentage of the adenosine diameter, the initial diameter (mean 88.8%) is significantly less than nitroglycerin (97.7%), and the diameter observed after ACh challenge (83.8%) is significantly smaller than either nitroglycerin or initial, consistent with an active vasoconstrictor response.

Although limited by small numbers, the comparison of ACh responses in patients who had either CMV infection or rejection versus those who had neither still approached statistical significance, suggesting that there may be exaggerated ACh-induced vasoconstriction consistent with more severely dysfunctional endothelium in those who experienced CMV infection or rejection. This observation requires further investigation in a larger series.

The data from intravascular ultrasound imaging and Doppler flow studies allow direct calculation of volume flow and CFR in the coronary branch vessel under consideration. Our data show a CFR of 5.06 in these heart transplant patients in response to adenosine, which is consistent with the CFR in normal individuals with dipyridamole and papaverine. In striking contrast to the active vasoconstriction produced in the epicardial conduit vessels provoked by ACh, mean coronary flow increased from 63.1 to 204 ml/min after ACh, consistent with vasodilation of the microvascular resistance vessels. These findings suggest that the endothelial injury that occurs after heart transplantation may be targeted toward larger vessels and may spare the microvasculature.

In summary, these data demonstrate consistent significant epicardial coronary vasoconstriction and an increase in coronary blood flow in response to ACh at 1 year after cardiac transplantation in a group of patients who have normal CFR in response to adenosine and no.
anatomic evidence of cardiac allograft vasculopathy. The data are consistent with the hypothesis that coronary artery endothelial dysfunction occurs frequently in heart transplant recipients. Endothelial dysfunction may be more severe in those who have experienced CMV infection or required parenteral steroid therapy for rejection episodes. The preservation of CFR suggests that the microvasculature may be spared in this process. The direct cause or causes of endothelial injury, including mechanical or thermal trauma, drug effects, infection, or immunologically mediated injury, remain uncertain.

Acknowledgment

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References

Endothelial dysfunction early after heart transplantation. Assessment with intravascular ultrasound and Doppler.
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