Impaired Coronary Dilator Responses to Substance P and Impaired Flow-Dependent Dilator Responses in Heart Transplant Patients With Graft Vasculopathy

ANDREAS MÜGGE, MD, FACC, BERND HEUBLEIN, MD, MICHAELA KUHN, MD,*
CARSTEN NOLTE, MD, AXEL HAVERICH, MD, JÖRG WARNECKE, BSc,
WOLF-GEORG FORSSMANN, MD,* PAUL R. LICHTLEN, MD, FACC
Hannover, Germany

Objectives. Because pathologic mechanisms for transplant vasculopathy are still uncertain, we tested the hypothesis that endothelial function, in terms of the release of endothelium-derived relaxing factor (EDRF), is impaired in patients with evidence of angiographic transplant vasculopathy.

Background. The long-term prognosis after heart transplantation is mainly determined by the development of transplant vasculopathy.

Methods. The study included 23 patients undergoing diagnostic cardiac catheterization approximately 40 months after heart transplantation. Patients were classified into those with (n = 8) and those without (n = 15) angiographic evidence of transplant vasculopathy. Coronary flow velocity (by intravascular Doppler echocardiography) and epicardial coronary diameter (by quantitative angiography) were determined after intracoronary bolus injections (1 ml) of the endothelium-dependent dilator substance P (20 pmol) and the endothelium-independent dilators nitroglycerin (0.1 mg) and papaverine (8 mg). Substances were injected through the lumen of the Doppler catheter, which was placed into the midportion of the left anterior descending artery.

Results. Increases in blood flow velocity in response to substance P were significantly less in patients with than in patients without evidence of transplant vasculopathy. In addition, flow-mediated dilation of epicardial coronary arteries in response to papaverine was abolished in patients with such evidence. Vasodilation of epicardial coronary arteries in response to nitroglycerin and increases in flow velocity in response to papaverine were similar in both groups.

Conclusions. These results suggest that transplant vasculopathy in heart transplant patients is associated with endothelial dysfunction (that is, impaired EDRF-mediated vasodilation). Furthermore, responsiveness of epicardial arteries to increased flow appears to be abolished in patients with evidence of transplant vasculopathy. These abnormal vascular functions may contribute to the pathogenesis of transplant vasculopathy and its vascular complications.

(J Am Coll Cardiol 1993;21:163-70)
vascular tone: relaxation due to the release of EDRF and constriction due to direct smooth muscle effects. Thus, acetylcholine may not be the best substance to test EDRF release in vivo. In fact, several studies (10–14) on the effect of intracoronary acetylcholine reported vasoconstriction as the principal response, irrespective of the presence or absence of angiographic signs of atherosclerosis. In agreement with the in vivo studies, acetylcholine also produced vasoconstriction in isolated human coronary arteries that appeared histologically free of atherosclerotic lesions (15).

Substance P appears to be more selective than acetylcholine in modulating vascular tone by the release of EDRF. In vitro relaxation induced by substance P in human coronary arteries is strongly dependent on the presence of intact endothelial cells; furthermore, substance P does not directly affect coronary smooth muscle tone (6,7,15). In vivo, intracoronary substance P induced dilation of epicardial coronary arteries as well as a reduction in coronary vascular resistance (16). Recently, substance P has also been shown to dilate epicardial coronary arteries in cardiac transplant patients with angiographically "normal" coronary arteries (17).

In the present study, we reevaluated the hypothesis that transplant vasculopathy in heart transplant patients is associated with endothelial dysfunction (that is, impaired EDRF-mediated vasodilation). However, in contrast to previous studies, we used substance P, nitroglycerin, and papaverine to test endothelial and smooth muscle function in heart transplant patients with and without transplant vasculopathy. Endothelial function was determined by measuring changes in coronary flow velocity and epicardial coronary artery diameter during cardiac catheterization.

### Methods

**Study patients (Table 1).** Twenty-three patients undergoing routine diagnostic cardiac catheterization were studied 39.9 ± 4.1 months (mean ± SEM) after heart transplantation. Heart transplantation was performed for ischemic heart disease in 6 patients, for idiopathic cardiomyopathy in 16 and for a malignant cardiac tumor in 1 patient. Patients were classified into patients with (n = 8) and without (n = 15) transplant vasculopathy on the basis of the diagnostic coronary angiograms that were independently reviewed by two cardiologists. Transplant vasculopathy was defined according to Gao et al. (18). Type A consists of focal eccentric lumen narrowing or obstruction involving the proximal epicardial vessels; type C = irregular proximal and distally diseased distal vessels.

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Pt. No.</th>
<th>Age (yr)/ Gender</th>
<th>Months After HTx</th>
<th>TVP Type</th>
<th>Donor Age (yr)/ Gender</th>
<th>Preexisting Cardiac Disease</th>
<th>Treated</th>
<th>Lipids (mmol/liter)</th>
<th>Isometric Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41/F</td>
<td>23</td>
<td>A</td>
<td>20/M</td>
<td>DCM</td>
<td>5</td>
<td>Chol: 7.54</td>
<td>Isometric: 150</td>
</tr>
<tr>
<td>2</td>
<td>44/M</td>
<td>45</td>
<td>C</td>
<td>22/M</td>
<td>ICM</td>
<td>9</td>
<td>TG: 2.75</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>47/M</td>
<td>14</td>
<td>A</td>
<td>24/M</td>
<td>ICM</td>
<td>5</td>
<td>Chol: 4.37</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>52/M</td>
<td>65</td>
<td>C</td>
<td>20/M</td>
<td>DCM</td>
<td>8</td>
<td>TG: 0.76</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>50/M</td>
<td>60</td>
<td>A</td>
<td>14/M</td>
<td>ICM</td>
<td>5</td>
<td>Chol: 7.14</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>58/M</td>
<td>57</td>
<td>C</td>
<td>41/F</td>
<td>DCM</td>
<td>7</td>
<td>TG: 1.71</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>34/M</td>
<td>39</td>
<td>C</td>
<td>17/M</td>
<td>DCM</td>
<td>3</td>
<td>Chol: 7.8</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>90/M</td>
<td>12</td>
<td>A</td>
<td>40/M</td>
<td>ICM</td>
<td>2</td>
<td>TG: 1.99</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>49/F</td>
<td>25</td>
<td>A</td>
<td>20/M</td>
<td>DCM</td>
<td>5</td>
<td>Chol: 6.52</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>30/M</td>
<td>24</td>
<td>A</td>
<td>20/M</td>
<td>DCM</td>
<td>6</td>
<td>TG: 1.37</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>18/M</td>
<td>16</td>
<td>A</td>
<td>19/M</td>
<td>DCM</td>
<td>2</td>
<td>Chol: 1.93</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>27/F</td>
<td>38</td>
<td>A</td>
<td>24/M</td>
<td>DCM</td>
<td>3</td>
<td>TG: 2.01</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>50/M</td>
<td>13</td>
<td>A</td>
<td>28/M</td>
<td>DCM</td>
<td>4</td>
<td>Chol: 1.87</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>60/M</td>
<td>39</td>
<td>A</td>
<td>22/M</td>
<td>ICM</td>
<td>8</td>
<td>TG: 1.78</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>49/M</td>
<td>45</td>
<td>A</td>
<td>31/M</td>
<td>DCM</td>
<td>6</td>
<td>Chol: 8.14</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>43/M</td>
<td>64</td>
<td>A</td>
<td>32/F</td>
<td>DCM</td>
<td>4</td>
<td>TG: 2.34</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>52/M</td>
<td>61</td>
<td>A</td>
<td>25/M</td>
<td>DCM</td>
<td>0</td>
<td>Chol: 6.24</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>50/M</td>
<td>50</td>
<td>A</td>
<td>21/M</td>
<td>DCM</td>
<td>7</td>
<td>TG: 1.69</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>40/F</td>
<td>69</td>
<td>A</td>
<td>19/F</td>
<td>DCM</td>
<td>8</td>
<td>Chol: 5.52</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>36/M</td>
<td>16</td>
<td>A</td>
<td>18/M</td>
<td>DCM</td>
<td>4</td>
<td>TG: 5.79</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>56/M</td>
<td>66</td>
<td>A</td>
<td>28/M</td>
<td>DCM</td>
<td>9</td>
<td>Chol: 5.00</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>35/M</td>
<td>49</td>
<td>A</td>
<td>18/M</td>
<td>Tumor</td>
<td>5</td>
<td>TG: 3.55</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>53/M</td>
<td>28</td>
<td>A</td>
<td>31/M</td>
<td>DCM</td>
<td>7</td>
<td>Chol: 6.03</td>
<td></td>
</tr>
</tbody>
</table>

Plasma lipid values were averaged for the posttransplantation period. Chol = total plasma cholesterol; DCM = diffuse coronary disease; F = female; HTx = heart transplantation; ICM = ischemic cardiomyopathy; M = male; Pt = patient; TG = total plasma triglycerides; Tumor = malignant cardiac tumor; TVP = transplant vasculopathy; type A = focal eccentric lumen narrowing or obstruction involving the proximal epicardial vessels; type C = irregular proximal and distally diseased distal vessels.
arteries involved. Type C is characterized by irregular proximal and diffusely diseased distal vessels. The lesions in four of the eight patients with transplant vasculopathy consisted of focal eccentric lumen narrowing of the right coronary artery (n = 2), left circumflex artery (n = 1) and the midportion of the left anterior descending artery (n = 1, stenosis <50%); in the remaining four patients with transplant vasculopathy, diffuse lesions were seen in the right (n = 1) and the left circumflex (n = 3) coronary artery. At the time of routine annual cardiac catheterization, all patients were without evidence of acute cardiac rejection in endomyocardial biopsy samples. Patients remained on their immunosuppressive medication (cyclosporine, steroids, azathioprine). Additional cardiac medication (angiotensin-converting enzyme inhibitors) was similar in both groups and was continued. No patient was taking nitrates or calcium antagonists.

Study design. Written informed consent was obtained from all patients before cardiac catheterization in accordance with guidelines of our local Ethical Committee. Diagnostic right and left heart catheterization, including right ventricular endomyocardial biopsy and coronary angiography, was performed by standard techniques. An 8F Gensini catheter with side holes was placed into the midportion of the coronary venous sinus just distal to the anterior interventricular vein. Before completion of coronary angiography, an additional 5,000 U of intracoronary heparin was given and an 8F guiding catheter (Malinckrodt) was positioned in the ostium of the left coronary artery. A 20-MHz pulsed Doppler infusion catheter (3F, Millar Instruments) was advanced over a 0.012-in. (0.03 cm) guide wire through the guiding catheter into the proximal segment of the left anterior descending artery. The Doppler catheter was carefully positioned to obtain a stable flow velocity signal. The guide wire was then withdrawn and the guiding catheter tip removed from the coronary ostium during flow measurements. The range-gate control was then adjusted to optimize the audio flow velocity signal and the phasic flow velocity waveform; the range-gate control was not changed thereafter. This technique has been previously shown to allow continuous reliable measurements of blood flow velocity (8, 19, 20).

Five minutes after control angiography, serial bolus injections (1 ml) of substance P (20 pmol dissolved in saline solution), nitroglycerin (0.1 mg diluted in saline solution), saline solution control and papaverine (8 mg diluted in saline solution) were administered in 6-min intervals through the central lumen of the Doppler catheter. The time of bolus injection lasted <5 s (13), and 50 s after each intervention hand injections of nonionic contrast material for coronary angiography (Ultravist, Schering AG) were repeated. Angiography was performed after velocity measurements to avoid the confusing effect of contrast medium on coronary flow. Throughout the bolus injections, heart rate, blood pressure and phasic and mean coronary flow velocities were continuously recorded. Bolus injections were well tolerated by all patients. The time interval of approximately 5 min between each intervention appeared to be sufficient to allow hemodynamic equilibration to baseline values, as judged by blood pressure and coronary blood flow velocity. In six patients with angiographically normal coronary arteries, additional pharmacologic interventions were performed 10 to 15 min before the serial bolus injections. In these patients, intracoronary saline solution followed by substance P (20 pmol/min) was infused through the Doppler catheter lumen for 2 min at an infusion rate of 1 ml/min. Blood samples (2.7 ml drawn in 3.6 mmol/liter of EDTA and 1 mmol/liter of M&B 22.948) were collected from the coronary sinus at 0, 30, 60, 90 and 120 s during the infusion of saline solution and substance P. An additional blood sample was obtained 30 s after stopping the infusion. These blood samples were used to measure platelet cyclic guanosine 5'-monophosphate (GMP) content.

Quantitative coronary angiography. Coronary angiography was performed with a simultaneous biplane LARC system (Phillips). The left anterior descending artery was positioned near the isocenter. Biplane 35-mm cineangiograms were recorded at a rate of 25 frames/s. End-diastolic angiograms were evaluated by a computer-assisted edge detection system (Cardiovascular Angiography Analysis System), as described previously (21). Coronary artery diameter was calibrated on the cine frame by comparison of the tip of the coronary catheter with a precision caliper immediately after angiography (21). Selection of optimal cine frames and coronary segments was performed by an experienced cardiologist without knowledge of the patients' drug regimen and clinical data. For each intervention, three segments (1 to 2-cm nonbranching segment) were analyzed: one segment of the circumflex artery for reference purposes and one segment each proximal and distal to the tip of the Doppler catheter. The proximal segment was not exposed to the injected substances and served to determine flow-dependent changes in coronary artery diameter. The distal segment was exposed to the substances injected through the lumen of the Doppler catheter. Vessels were assumed to have a circular cross section, and area was calculated as \( \pi r^2 \).

Measurement of platelet cyclic GMP content. Blood samples drawn from the coronary venous sinus were centrifuged (200 g, 10 min, 4°C). Platelet-rich plasma was collected and recentrifuged (400 g, 10 min, 4°C). The supernatants were discarded and pellets were resuspended in 0.5 ml of ice-cold ethanol (70% in distilled water) and agitated for 1 min. After centrifugation (2,000 g, 10 min, 4°C), supernatants were dried overnight under vacuum. Pellets were dissolved in sodium acetate (50 mmol/liter), and the cyclic GMP content of the samples was measured by radioimmunoassay, as described previously (22).

Material. Drugs were obtained from the following companies: Substance P from Clinalfa, Basel, Switzerland; nitroglycerin (Peringanit) from Schwarz Pharma, Monheim, Germany; papaverine (Paveron) from Karlsharma, Karl-
Figure 1. Effect of intracoronary substance P (20 pmol) and vehicle (0.9% sodium chloride [NaCl]) on blood flow velocity (a), epicardial coronary artery diameter (b) and mean arterial pressure (c) in patients after heart transplantation. Substance P was injected through a Doppler catheter that was placed into the midportion of the left anterior descending artery (LAD). Mean arterial pressure and coronary flow velocity were measured before and 10 to 50 s after substance P injection. Coronary artery diameter (proximal [Prox.] and distal to the tip of the Doppler catheter; circumflex artery as a reference [Ref.] segment) was measured by quantitative angiography 50 s after substance P injection. Intracoronary injection of saline solution had no effect on coronary artery diameter. Experiments were performed in 15 patients without angiographic signs of transplant vasculopathy (TVP neg) and 8 patients with angiographically proved transplant vasculopathy (TVP pos). Values are mean value ± SEM; asterisks denote significant differences between patients with and without angiographic evidence of transplant vasculopathy.

Figure 2. Effect of intracoronary nitroglycerin (0.1 mg) on blood flow velocity (a), epicardial coronary artery diameter (b) and mean arterial pressure (c) in patients after heart transplantation. Nitroglycerin was injected through a Doppler catheter that was placed into the midportion of the left anterior descending artery (LAD). Mean arterial pressure and coronary flow velocity were measured before and 10 to 50 s after nitroglycerin injection. Coronary artery diameter (proximal and distal to the tip of the Doppler catheter) was measured 50 s after nitroglycerin injection by quantitative angiography. Experiments were performed in 15 patients without angiographic signs of transplant vasculopathy (TVP neg) and 8 patients with angiographically proved transplant vasculopathy (TVP pos). Values are mean value ± SEM; asterisks denote significant differences between patients with and without angiographic evidence of transplant vasculopathy.

Results

Eight of 23 patients showed angiographic evidence of transplant vasculopathy (Table I). Patients with and without transplant vasculopathy did not differ with regard to the underlying heart disease before heart transplantation, number of treated rejection periods, ischemic time or plasma cholesterol levels.

Substance P (Fig. 1). Injection of vehicle (saline solution) through the Doppler catheter lumen caused a transient minimal (16%) increase in left anterior descending artery blood flow velocity, which was almost normalized to baseline within 15 s after bolus injection (Fig. 1a). In patients without evidence of transplant vasculopathy, substance P maximally increased coronary flow velocity by approximately 117%, with a peak increase 10 to 20 s after a bolus injection that lasted for 40 s (Fig. 1a). In patients with evidence of transplant vasculopathy, the substance P-induced increase in peak to baseline flow velocity was significantly impaired (117 ± 17% vs. 38 ± 12%, p < 0.01) (Fig. 1a). Epicardial coronary artery diameter measured distal to the tip of the Doppler catheter increased in response to substance P by 11.7 ± 3.0% in patients without transplant vasculopathy and by 5.3 ± 3.8% in patients with transplant vasculopathy (p = 0.06) (Fig. 1b). Epicardial coronary artery diameter measured proximal to the tip of the Doppler catheter increased after substance P injection by 4.5 ± 3.1% and 0 ± 1.9%, respectively, in patients without and with transplant vasculopathy (p = NS) (Fig. 1b). The reference segment (circumflex coronary artery) showed almost no change in diameter after substance P injection in both groups (Fig. 1b). Beginning 20 s after bolus injection (Fig. 1c), intracoronary substance P injection slightly reduced mean arterial pressure in both groups.

Nitroglycerin (Fig. 2). In both patient groups, nitroglycerin produced a biphasic response on coronary flow velocity; first a short-lasting increase, then a decrease in peak to
Figure 3. Effect of intracoronary papaverine (8 mg) on blood flow velocity (a), epicardial coronary artery diameter (b) and mean arterial pressure (c) in patients after heart transplantation. Papaverine was injected through a Doppler catheter that was placed into the midportion of the left anterior descending artery (LAD). Mean arterial pressure and coronary flow velocity were measured before and 10 to 50 s after papaverine injection. Coronary artery diameter (proximal and distal to the tip of the Doppler catheter) was measured 50 s after papaverine injection by quantitative angiography. Experiments were performed in 15 patients without angiographic signs of transplant vasculopathy (TVP neg) and 8 patients with angiographically proved transplant vasculopathy (TVP pos). Values are mean value ± SEM; asterisk denotes significant differences between patients with and without angiographic evidence of transplant vasculopathy.

baseline flow (Fig. 2a). This short-lasting increase in peak to baseline flow was somewhat less in patients with than in patients without evidence of transplant vasculopathy when evaluated 10 s after bolus injection (80 ± 22% vs. 25 ± 5%, p < 0.05) (Fig. 2a). Epicardial coronary artery diameter measured distal to the tip of the Doppler catheter increased in response to nitroglycerin by 16.3 ± 4.2% in patients without and 14.1 ± 2.6% in patients with transplant vasculopathy (p = NS) (Fig. 2b). Epicardial coronary artery diameter measured proximal to the tip of the Doppler catheter increased after nitroglycerin injection by 6.5 ± 2.1% and by 2.0 ± 2.8%, respectively, in patients without and with transplant vasculopathy (p = NS) (Fig. 2b). Intracoronary nitroglycerin injection reduced mean arterial pressure to a similar extent in both groups (Fig. 2c).

Papaverine (Fig. 3). In both groups of patients, intracoronary papaverine produced a pronounced increase in coronary flow velocity by approximately 300%, which was at its maximum at 30 s after bolus injection (Fig. 3a). This increase in peak to baseline flow was of similar magnitude in patients with and without evidence of transplant vasculopathy (304 ± 39% vs. 336 ± 46%, respectively, p = NS) (Fig. 3a). Epicardial coronary artery diameter measured distal to the tip of the Doppler catheter increased in response to papaverine by 18.2 ± 4.0% in patients without and 16.5 ± 3.0% in patients with transplant vasculopathy (p = NS) (Fig. 3b). Epicardial coronary diameter measured proximal to the tip of the Doppler catheter increased after papaverine injection by 13.5 ± 3.8% and 0.4 ± 3.9% in patients without and with transplant vasculopathy, respectively (p < 0.01) (Fig. 3b). Intracoronary papaverine injection slightly reduced mean arterial pressure to a similar extent in both groups (Fig. 2c).

Figure 4. Plot of the percent change in platelet cyclic guanosine 5'-monophosphate (GMP) content of blood samples obtained from the coronary venous sinus during intracoronary infusion of substance P (10 nmol/min for 2 min). Experiments were performed in six patients with angiographically normal coronary arteries after heart transplantation (no evidence of transplant vasculopathy). Basal cyclic GMP content was 0.23 ± 0.078 pmol/sample. Substance P infusion caused significant increases in platelet cyclic GMP content as observed at 60 and 90 s after the start of infusion (*p < 0.05 vs. basal value). Thirty seconds after stopping the infusion, platelet cyclic GMP content had already declined to basal levels. Infusion of vehicle (saline solution) did not significantly affect platelet cyclic GMP content. Values are mean value ± SEM.

Discussion

Several observations suggest that mechanisms of transplant vasculopathy are different from those of common atherosclerosis. Transplant vasculopathy affects transplant recipients without typical cardiovascular risk factors whose own heart had no coronary artery disease (2). Several pathogenic mechanisms for transplant vasculopathy have been proposed (1), including endothelial damage through cytomegalovirus infection (23), immunologic incompatibility
between donor and recipient (24) and antibody-mediated rejections (25).

In this study, we tested the hypothesis that endothelial damage is present in patients with transplant vasculopathy. Endothelial function was tested by measuring vascular responses to intracoronary substance P, an endothelium-dependent vasodilator, and changes in blood flow after intracoronary administration of papaverine. Our results show that the vasodilator response of the microvasculature to intracoronary substance P is significantly impaired in patients with coronary graft vasculopathy compared with the response in transplant patients with angiographically normal coronary arteries. In addition, flow-mediated dilation of epicardial coronary arteries in response to papaverine appears to be abolished in patients with transplant vasculopathy. In contrast, vasodilation of epicardial arteries in response to intracoronary nitroglycerin and an increase in coronary flow velocity in response to intracoronary papaverine were similar in transplant patients with and without transplant vasculopathy. These results suggest that transplant vasculopathy in transplant patients is associated with endothelial dysfunction (that is, impaired EDRF-mediated vasodilation).

Limitations of study. Substance P is a neuropeptide found in sensory neurons of the peripheral nervous system, vagus, some sympathetic ganglia and the perivascular nerves of small arteries in the human heart (17,26). In isolated human coronary arteries, substance P is a potent and specific stimulator of EDRF release (6,7,15,27,28). In contrast to acetylcholine, substance P has no direct effects on coronary smooth muscle tone (6,7,14,28). Thus, it may be more suitable than acetylcholine for studying EDRF-mediated vascular responses in the human coronary circulation. Conversely, dilator responses to substance P in human epicardial coronary arteries in vitro are known to be transient because of a tachyphylaxis phenomenon (7,15). Even one concentration of substance P causes tachyphylaxis, at least under in vitro conditions (7,15). It is unknown whether this phenomenon is restricted to large epicardial arteries or involves both large and resistance coronary arteries. Because of this phenomenon, we used bolus injections of substance P instead of infusions in the present study. This study design may have certain limitations. Although quantitative angiography was performed shortly (50 s) after bolus injection, changes in coronary diameter might not be measured at steady state conditions. This limitation appears to be of minor importance for relatively long-acting substances (for example, nitroglycerin and papaverine). In fact, quantitative angiography revealed substantial changes in coronary artery diameter in response to nitroglycerin and papaverine even 50 s after the start of the bolus injection that were of similar magnitude to those in other studies using intracoronary infusion protocols (13,16,17,29). This limitation, however, may have certain implications for short-acting drugs such as substance P. At the time of quantitative angiography, substance P-induced increases in coronary flow velocity were almost normalized to baseline values. Thus, measurements 30 s after bolus injection may slightly underestimate the vasodilator effects of substance P on large epicardial arteries. It is reasonable to assume that this underestimation of the vasodilator effect of substance P will occur in patients with or without transplant vasculopathy. Thus, this potential systematic error is not likely to interfere with the main emphasis of the present study to compare vasodilator responses between both group of patients.

Platelet cyclic GMP content. In six patients without evidence of transplant vasculopathy, we also tested the hypothesis that intracoronary substance P does stimulate the release of EDRF, which is known to increase cyclic GMP content in platelets in vitro, thereby inhibiting platelet aggregation (30–32). In agreement with these in vitro observations, stimulation of EDRF has also been reported (33) to increase cyclic GMP levels in washed platelets during passage through the coronary vascular bed of isolated rabbit hearts. A similar phenomenon could be demonstrated in the present study. During infusion of substance P, cyclic GMP content in the plasma-rich fraction of blood samples drawn from the coronary sinus increased approximately 60%. Incubation of whole blood with substance P did not affect platelet cyclic GMP content. Furthermore, infusion of vehicle (saline solution) had no effect on platelet cyclic GMP content. These preliminary findings are consistent with the hypothesis that intracoronary substance P stimulates the release of EDRF and that luminally released EDRF increases the cyclic GMP content in circulating platelets in humans. Although it was not the purpose of the present study to characterize this phenomenon further, this observation might contribute to our understanding of the effects of substance P in patients without evidence of transplant vasculopathy and the results observed in patients with such evidence. In addition, it might form the basis for a biochemical test using circulating platelets as an indirect indicator of endothelial function in patients with transplant vasculopathy or coronary artery disease.

Comparison with previous studies. Crossman et al. (16) demonstrated an increase in epicardial coronary artery diameter and a significant increase in the coronary sinus blood oxygen saturation in response to intracoronary substance P in 13 patients with angiographically normal coronary arteries. These results suggest that intracoronary substance P dilates both large arteries as well as resistance vessels. Kushwaha et al. (17) demonstrated in 12 heart transplant patients without transplant vasculopathy a significant increase in epicardial coronary artery diameter that was similar in magnitude to that achieved with isosorbide dinitrate. These results suggest that endothelial function as assessed by vascular responses to substance P is principally preserved in heart transplant recipients with angiographically normal coronary arteries. In agreement, our study also demonstrated a substance P-induced increase in epicardial coronary artery diameter and a reduction in coronary vascular resistance in patients without transplant vasculopathy.
suggesting that substance P dilates both large and small (resistance) coronary arteries. Because intracoronary substance P increased coronary flow, changes in epicardial coronary artery diameter may be mediated indirectly by increased flow, as well as through direct effects on the vessel wall. In the present study, the distal but not the proximal segment of the left anterior descending artery was exposed to drugs. Thus, changes in diameter of this proximal segment can be assumed to be flow mediated (34). In our study, in contrast to the distal left anterior descending artery segment, the proximal diameter of this vessel did not increase significantly in response to substance P, although coronary blood flow velocity increased by about twofold in patients without evidence of transplant vasculopathy. This finding suggests that the increase in the diameter of the distal coronary segment in response to substance P is mainly drug mediated and not flow dependent. In contrast, the increase in coronary flow velocity in response to substance P probably was not sufficient to cause flow-dependent dilation. In comparison, intracoronary papaverine in the same patients increased coronary flow velocity by about fourfold and this led to a significant (approximately 18%) increase in proximal coronary artery diameter.

In patients with evidence of transplant vasculopathy, dilation of the distal left anterior descending artery segment in response to substance P was attenuated compared with that in patients without such evidence. Furthermore, peak to baseline flow velocity in response to substance P was significantly reduced in patients with transplant vasculopathy. This reduced responsiveness of epicardial and resistance coronary arteries to substance P was not due to a general impairment of the relaxing mechanisms. In the same patients, maximal epicardial coronary dilation in response to nitroglycerin and maximal reduction in regional left anterior descending artery resistance in response to papaverine were preserved. These results suggest that selective vascular responses to substance P are impaired in patients with transplant vasculopathy. This corelease of vasoconstrictors would also result in impaired vasodilation in response to substance P. The mechanism, however, appears to be unlikely because substance P does not elicit vasodilatation in vitro (28) or in vivo after inhibition of EDRF release with L-arginine analogues (35).

Intracoronary nitroglycerin injections produced a small transient increase in blood flow velocity that was somewhat less in patients with evidence of transplant vasculopathy compared with control patients. The mechanism of this transient response to intracoronary nitroglycerin is not known. Thus, it remains unclear whether this impaired effect of intracoronary nitroglycerin on blood flow velocity in patients with transplant vasculopathy is related to endothelial dysfunction.

Abolished flow-mediated vasodilation. A second major finding of the present study is that flow-mediated dilation of epicardial coronary arteries is abolished in patients with evidence of transplant vasculopathy. Mechanisms of flow-mediated vasodilation are complex and involve opening of ion channels and release of humoral vasoconstrictors and vasodilators (36). Flow-mediated dilation of larger arteries appears to be dependent on intact endothelial cells and probably involves the release of EDRF (37,38). Thus, the lack of flow-mediated vasodilation of epicardial coronary arteries in patients with evidence of transplant vasculopathy would be consistent with the hypothesis that transplant vasculopathy is associated with endothelial dysfunction (that is, reduced release of EDRF). Similar to the present findings in patients with evidence of transplant vasculopathy, impaired flow-dependent relaxation has been described in patients with coronary artery disease. Whereas the peak increases in blood flow in response to adenosine (39) or papaverine (40) were similar in patients with angiographically normal or diseased coronary arteries, dilation of a proximal coronary artery segment that had been exposed to increased flow but not to the drugs was virtually abolished in patients with atherosclerosis. The precise mechanism of impaired flow-mediated dilation in patients with coronary artery disease is unknown.

Impaired vascular responsiveness to intracoronary substance P and loss of flow-vasodilation of epicardial human coronary arteries might be signs of one pathogenic mechanism in patients with transplant vasculopathy. Both phenomena could be related to impaired release of EDRF. It remains unclear why certain patients elicit these vascular abnormalities after heart transplantation. At present, a primary immunologic stimulus, including chronic regional immune responses and stimulation of inflammatory cells, is the most likely explanation for the development of transplant vasculopathy (1). The link between immune responses/stimulation of inflammatory cells and the hypothetized impaired EDRF release in patients with transplant vasculopathy is unknown; one might speculate that this phenomenon is related to the “oxidative stress” secondary to inflammatory cells, as was recently suggested in an animal model of atherosclerosis (41,42).

We gratefully appreciate the critical review of the manuscript by Keith Comess, MD.

References


