Intracoronary Ultrasound Assessment of Morphological and Functional Abnormalities Associated With Cardiac Allograft Vasculopathy

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Background The diffuse nature of cardiac allograft vasculopathy makes early detection of the disease by traditional noninvasive methods or coronary angiography difficult. The aim of this study was to determine if there is a relation between abnormalities in vessel wall morphology, as assessed by intracoronary ultrasound, and a decreased vasodilatory response to the endothelium-dependent vasodilator papaverine hydrochloride and if cardiac allograft vasculopathy detected by coronary angiography is associated with specific intracoronary ultrasound findings.

Methods and Results Twenty-three heart transplant recipients underwent 25 intracoronary ultrasound studies and 24 studies of coronary vasomotor tone 10 days to 8.3 years after surgery using a 20-MHz intracoronary ultrasound catheter. The studies were divided in two groups according to the presence (n=7, group 1) or absence (n=18, group 2) of angiographically evident cardiac allograft vasculopathy. Qualitative assessment of vessel wall morphology and quantitative analysis of the vasodilator response to the injection of papaverine hydrochloride into the coronary artery distal to the imaging site were performed off-line, and results for the two study groups were compared. A significantly higher percentage of patients with than without angiographic evidence of cardiac allograft vasculopathy had a three-interface vessel wall morphology by intracoronary ultrasound (100% versus 11%, P<.001). In two recipients who underwent two serial studies, the appearance of three interfaces in the vessel wall or a progressive thickening of the inner interface of the vessel wall was evident cardiac allograft vasculopathy. The vasodilator response to papaverine was less in patients with than in those without angiographically evident cardiac allograft vasculopathy both in terms of absolute and relative increases in lumen diameter (+0.1±0.12 mm versus +0.3±0.17 mm, P<.05, and +5.1±5.3% versus +8.2±5.3%, P=NS) and lumen cross-sectional area (+0.5±0.6 mm² versus +1.7±1.1 mm², P<.02, and +7.1±8.8% versus 16.6±11.0%, P=.055), respectively.

Conclusions Intracoronary ultrasound assessment of vessel wall morphology and evaluation of vascular response to endothelium-dependent vasodilators are useful techniques for detecting cardiac allograft vasculopathy. (Circulation. 1994;89:272-277.)

Key Words • transplantation • vasodilation

The diffuse nature of cardiac allograft vasculopathy makes early detection of the disease with noninvasive methods or coronary angiography difficult. Pathological evidence of cardiac allograft vasculopathy has been found in the absence of angiographically demonstrable disease. Intracoronary ultrasound imaging has been shown recently to detect vessel wall abnormalities even when the coronary arteries are angiographically normal. However, it has not been conclusively shown that the three-interface vessel wall morphology on intracoronary ultrasound imaging (inner echogenic layer believed to represent an abnormally thick intima, a dark medial layer, and an outer echogenic area with ill-defined external border) actually is a manifestation of either native or allograft coronary artery disease. Studies of vascular responses to endothelium-dependent and endothelium-independent vasodilators have been proposed as a useful method for the early detection of cardiac allograft vasculopathy. Vasoconstriction in response to acetylcholine has provided circumstantial evidence of endothelial dysfunction in heart transplant recipients but acetylcholine-induced vasoconstriction has not consistently distinguished patients with from those without cardiac allograft vasculopathy. In contrast, vascular responses to endothelium-independent vasodilators such as nitroglycerin remain normal regardless of the presence or absence of three-interface vessel morphology on intracoronary ultrasound imaging. Papaverine hydrochloride increases flow by vasodilating small intramyocardial resistance vessels. It is hypothesized that papaverine-induced vasodilatation is due to the release of endothelium-derived relaxing factor (EDRF) stimulated by shear stress on the vessel wall endothelium. Calcium channels activated by shear stress may be the mediators of EDRF release. Vasodilatation in response...
to papaverine is diminished in patients with angiographically detectable, nonobstructive native coronary artery disease.\textsuperscript{8,17} The study of vascular luminal size changes in response to endothelium-dependent vasodilators on intravascular ultrasound images may unmask vascular abnormalities absent at baseline conditions on both angiogram and intracoronary ultrasound. The objective of this study, therefore, was to evaluate the relation between vessel wall morphology and papaverine-mediated vasodilatation assessed by intracoronary ultrasound and angiographic evidence of cardiac allograft vasculopathy.

**Methods**

**Patient Population**

Twenty-three heart transplant recipients (17 men and 6 women; age, 49 ± 12 years [±SD]; range, 21 to 66 years) underwent 25 intracoronary ultrasound studies 10 days to 8.3 years after surgery. In 24 studies, intracoronary ultrasound images were obtained before and after intracoronary injection of papaverine. Mean donor age was 27 ± 9 years (range, 14 to 45). Studies were divided into two groups according to the presence (n = 7, group 1) or absence (n = 18, group 2) of angiographically evident cardiac allograft vasculopathy defined as focal stenosis, distal pruning, or progressive tapering of the epicardial coronary arteries.\textsuperscript{9} All recipients were free of acute cellular rejection at the time of the intracoronary ultrasound study.

**Study Protocol**

Written informed consent for participation in the study was obtained from all patients before diagnostic coronary angiography according to the guidelines set forth by the Institutional Review Board for the Protection of Human Subjects at Loyola University Medical Center. Vasoactive drugs were withheld for at least 12 hours before cardiac catheterization. Diagnostic coronary angiography was performed using the femoral approach and standard techniques. Patients received 2000 U of heparin intravenously immediately after cannulation of the femoral artery and an additional 8000 U of heparin intravenously before insertion of the 20-MHz intracoronary ultrasound catheter (Endosonics Corp, Pleasanton, Calif). Optimal angiographic views were chosen to maximize visualization of each coronary segment in orthogonal projections. For those two patients who underwent serial evaluations, the same angiographic views were used for each study. An 8F guiding catheter was introduced into the descending aorta. A 5F (n = 8) or 3.5F (n = 17) intracoronary ultrasound catheter was advanced through the lumen of the guiding catheter until it protruded beyond the guiding catheter into the ascending aorta. Image subtraction of the intracoronary ultrasound catheter was done on-line with a software program (Endosonics Corp) incorporated in the computer hardware. The intracoronary ultrasound catheter was retrieved inside the guiding catheter. The guiding catheter was then advanced and positioned in the ostium of the coronary artery selected for study by intracoronary ultrasound based on ease of access, vessel size, and tortuosity. The left anterior descending coronary artery was selected in 22 studies, the circumflex in 3 studies. A 0.014-inch guide wire was advanced through the central lumen of the intracoronary ultrasound into the chosen vessel, and the intracoronary ultrasound was then advanced over the wire. While recording ultrasonic images on super VHS videotape, standard cineangiograms were obtained to accurately localize ultrasonic imaging sites. After the length of the coronary artery was visualized, a site was chosen based on image resolution, central catheter position, and maximal visualization of the vessel wall for imaging and assessment of vasomotor tone responses. The intracoronary ultrasound position as well as the gain and gray scale settings were kept constant during the study of vasomotor tone.

After baseline images were recorded, papaverine hydrochloride (7.5 mg) was administered through the central lumen of the intracoronary ultrasound catheter, distal to the imaging site, to evaluate flow-dependent rather than local papaverine-induced vasodilatation. Ultrasound images of papaverine-induced changes in vessel size were continuously recorded. After return to baseline dimension, 200 \( \mu \)g of intracoronary nitroglycerin was administered, and angiographic views were repeated in the vasodilated state for comparison with follow-up angiographic studies. Nitroglycerin was not given before papaverine hydrochloride, since papaverine-induced, flow-mediated vasodilatation may not have been appreciated in maximally dilated vessels. The intracoronary ultrasound was removed, and the study was terminated.

**Data Analysis**

The presence or absence of angiographic cardiac allograft vasculopathy was determined by two independent observers. Qualitative and quantitative intracoronary ultrasound image analyses were performed off-line by the same two independent observers. Intracoronary ultrasound images were classified according to whether or not three distinct interfaces were detected in the vessel wall. Quantitative measurements of vessel luminal diameter and cross-sectional area were performed using a Rasterops video capture card (Santa Clara, Calif), which permits transfer of representative frames from the videocorder to the computer and the software program IMAGE 1.43 (National Institutes of Health, Research Services Branch, Bethesda, Md). A frame was chosen for subsequent measurements by both observers based on image resolution, 360° visualization of the vessel wall, and greatest luminal dimension. Each observer made two measurements of the long-axis and short-axis and cross-sectional area of the lumen before and after papaverine. The frames used for image analysis were those having the largest luminal dimensions at baseline and after papaverine injection.

**Statistical Analysis**

Correlation coefficients were calculated for intraobserver and interobserver measurements to assess their consistency and reproducibility (Table 1). The presence of three interfaces in the vessel wall on intracoronary ultrasound in patients with and without angiographically evident cardiac allograft vasculopathy was compared using the Fisher's exact test. Student's \( t \) tests were used to compare changes in vessel diameters and cross-sectional areas before and after intracoronary papaverine between the groups. Statistical significance was assumed if the null hypothesis could be rejected at the \( P = .05 \) level.

**Results**

**Reproducibility of Measurements**

The presence or absence of three interfaces of the vessel wall was agreed upon by both observers in all cases. Measurements by each observer and between observers were reproducible with a high correlation coefficient (Table 1).

**Qualitative Intracoronary Ultrasound Analysis of Vessel Wall Morphology**

Representative intracoronary ultrasound images from patients without and with cardiac allograft vasculopathy are shown in Fig 1. Three ultrasonic vessel wall interfaces were present in all recipients with angiographically evident cardiac allograft vasculopathy but in only 11% of recipients without angiographic cardiac
allograft vasculopathy (P<.001) (Fig 2). In the two recipients studied serially, the development of angiographic cardiac allograft vasculopathy occurred in parallel with the ultrasonic appearance of three vessel wall interfaces in one recipient and a measurable increase in thickness of the inner interface in the other recipient.

Quantitative Analysis of Vessel Luminal Dimensions

Representative studies of the vasodilatory response to the injection of intracoronary papaverine in recipients with and without angiographic evidence of cardiac allograft vasculopathy are shown in Fig 3. Baseline vascular luminal dimensions were similar in groups 1 and 2 (Table 2). The absolute increase in vascular luminal diameter was significantly less in patients with than in those without angiographically evident cardiac allograft vasculopathy (+0.1±0.12 mm versus +0.3±0.17 mm, P<.05) (Fig 4). Although the relative change in vascular luminal diameter was less in patients with than without angiographic cardiac allograft vasculopathy, the difference was not statistically significant (+8.2±0.11% versus 5.3±1.3%, P=NS) (Fig 5). The increase in vessel luminal cross-sectional area was less in patients with than without angiographic cardiac allograft vasculopathy in terms of both absolute (+0.5±0.6 mm² versus +1.7±1.1 mm², P<.02) (Fig 6) and relative luminal cross-sectional area changes (+7.1±8.8% versus +16.6±11.0%, P=.055) (Fig 7). Of interest, the vasomotor tone study done in one recipient without angiographic cardiac allograft vasculopathy but with three interfaces on intracoronary ultrasound showed absolute and relative changes in diameter (0.4 mm and 1.4%) and cross-sectional area (0.73 mm² and 9.3%) similar to the vascular luminal changes detected in patients with cardiac allograft vasculopathy.

Discussion

The results of our study indicate that there is a strong association between the presence of angiographically evident cardiac allograft vasculopathy and the appearance of three interfaces in the vessel wall by intracoronary ultrasound. All intracoronary ultrasound studies done in patients with angiographically visible cardiac allograft vasculopathy revealed a three-interface vessel wall morphology.
whereas only 11% of intracoronary ultrasound studies in patients without angiographically evident cardiac allograft vasculopathy showed this ultrasonic vessel wall abnormality. Our results also indicate that coronary vasodilatation in response to the endothelium-dependent vasodilator papaverine was blunted in patients with angiographically evident cardiac allograft vasculopathy. As previous studies have shown that papaverine-induced vasodilation is mediated by EDRF,\textsuperscript{13-15} the coexistence of abnormal vessel wall morphology and impaired papaverine-induced vasodilatation indirectly suggests that the three interfaces seen on intracoronary ultrasound may be attributable to intimal injury and a resultant decrease in EDRF production.

Similar findings have been seen in native coronary vessels.\textsuperscript{17} The observation that the response of allograft coronary arteries to papaverine is similar to that of native vessels with or without nonobstructive coronary
artery disease suggests that vascular responses to papaverine are not influenced by denervation. Vascular responses to acetylcholine, another endothelium-dependent vasodilator, have been shown to be abnormal early and late after transplantation even in the absence of angiographic cardiac allograft vasculopathy, perhaps reflecting the presence of endothelial damage and not necessarily that of cardiac allograft vasculopathy. In contrast, in our previous study done early after heart transplantation, vasodilatation of the coronary arteries in response to papaverine was uniformly normal and similar to that reported by other investigators in nontransplant patients with normal coronary arteries. In this study, the degree of papaverine-induced vasodilatation was significantly less in patients with than in those without angiographic evidence of cardiac allograft vasculopathy. In native atherosclerotic coronary arteries that vasoconstricted in response to acetylcholine, flow-mediated vasodilatation was preserved. The differential ability of papaverine and acetylcholine to identify patients with and without cardiac allograft vasculopathy may be due to the fact that these two endothelium-dependent vasodilators produce their vascular effects by means of distinct intermediate mediators. Muscarinic receptors on the endothelium may also be more sensitive to ischemic, immunologic, and other insults after transplantation. Thus, flow-mediated vasodilatation induced by papaverine may be more specific to detect cardiac allograft vasculopathy rather than changes due to endothelial abnormalities other than cardiac allograft vasculopathy.

The vasodilatation produced by papaverine is independent of flow within 30 seconds of injection and later becomes flow dependent. Because in our study the greatest changes in allograft coronary luminal size occurred in approximately 60 seconds (68.9±18 seconds; median, 63 seconds) after papaverine injection, maximal papaverine-induced vasodilatation probably is due to increased flow. Interestingly, flow-mediated coronary vasodilatation in one recipient without cardiac allograft vasculopathy but with three interfaces on intracoronary ultrasound demonstrated a blunted response to intracoronary papaverine similar to that observed in recipients with angiographic cardiac allograft vasculopathy. This finding suggests that the appearance of three interfaces on intracoronary ultrasound and a blunted vasodilatory response to papaverine may allow earlier detection of cardiac allograft vasculopathy than coronary angiography. These data are preliminary, and serial studies in a larger number of patients are in progress.

Vascular luminal cross-sectional area may more accurately evaluate vessel luminal dimension than diameter because diameter may not accurately reflect vessel size in the presence of luminal eccentricity. This may explain the clearer demonstration of the difference in flow-mediated vasodilatation, in both absolute and relative terms, with cross-sectional area rather than with diameter measurements.

Simultaneous flow measurements were not performed because of the technical limitations of our equipment. However, continuous on-line intracoronary ultrasound monitoring, done to detect the maximal response to papaverine, may have compensated at least partially for this limitation. Retrograde flow of papaverine proximal to the imaging site was unlikely because the intracoronary ultrasound catheter did not obstruct.

**Table 2. Baseline Vessel Luminal Dimensions**

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<thead>
<tr>
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<th>Angiographic CAV</th>
<th>No Angiographic CAV</th>
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<tbody>
<tr>
<td>Diameter, mm</td>
<td>3.3±0.4</td>
<td>3.6±0.8</td>
</tr>
<tr>
<td>Cross-sectional area, mm²</td>
<td>10.2±2.6</td>
<td>9.0±4.2</td>
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CAV indicates cardiac allograft vasculopathy.

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**Figure 5.** Bar graph. Relative change in vessel luminal diameter in response to papaverine (PAP) in patients with and without coronary allograft vasculopathy (CAV).
the coronary lumen, and antegrade flow in the coronary vessel probably continued during papaverine injection. Because computer software for gating phases of the cardiac cycles was not available at the time of the study, we used the largest vessel dimension before and after papaverine injection for consistency of data analysis. Because cardiac allograft vasculopathy tends to be more diffuse than native coronary artery disease, it is possible that coronary luminal changes occurring at the site visualized by intracoronary ultrasound may be representative of those occurring throughout the vessel. Further studies are required to determine if the changes observed at one site are representative of the entire vessel.

Our study is limited by the small patient population, the paucity of serial studies, the lack of direct measurements of flow, and the lack of gating of cardiac cycles. However, despite its limitations, our study shows a significant association between the morphological appearance and functional abnormalities of allograft coronary arteries. The combined use of intracoronary ultrasound to evaluate vessel wall morphology and flow-mediated vascular responses to papaverine may allow identification of cardiac allograft vasculopathy before it becomes angiographically apparent. Studies evaluating the diagnostic accuracy of this approach are in progress.

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References

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