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Effect of L-Arginine on Coronary Endothelial Function in Cardiac Transplant Recipients

Relation to Vessel Wall Morphology

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Background Coronary endothelial vasodilator dysfunction is a common finding in cardiac transplant recipients and may represent an early marker for the development of intimal thickening and graft atherosclerosis. The present study tested the hypothesis that endothelial dysfunction precedes intimal thickening and that administration of L-arginine, the precursor of endothelium-derived relaxing factor, improves endothelial vasodilator function of coronary conduit and resistance vessels if given at an early stage of graft atherosclerosis.

Methods and Results Acetylcholine (10^{-6}, 10^{-5}, 10^{-4} mol/L) was infused into the left anterior descending or circumflex artery and repeated after intravenous infusion of L-arginine ($10\ mg \cdot kg^{-1} \cdot min^{-1}$ over 20 minutes) in 18 cardiac transplant recipients. Epicardial responses were evaluated by quantitative angiography, and the microcirculation was studied by determination of coronary blood flow with a Doppler flow velocity wire. Intimal thickening was assessed by intravascular ultrasound (n=14). In epicardial coronary arteries, acetylcholine tended to elicit vasoconstriction. Epicardial coronary vasoconstriction elicited by acetylcholine was attenuated by infusion of L-arginine (10^{-4} mol/L, −6.8% versus −2.8%; $P<.01$); this beneficial effect was observed predominantly in patients with normal intravascular ultrasound characteristics. In coronary resistance vessels, acetylcholine induced vasodilation, reflected by increases in coronary blood flow. The acetylcholine-induced increase in blood flow was significantly enhanced with L-arginine (at a dose of 10^{-4} mol/L, +121% versus 176%; before versus after L-arginine, $P<.002$).

Conclusions The coronary vasculature of cardiac transplant recipients exhibits a generalized endothelial dysfunction of conduit and resistance vessels. L-Arginine improves endothelial dysfunction of both coronary microvasculature and epicardial coronary arteries. The reversibility of epicardial endothelial dysfunction by L-arginine is more likely in vessels with normal wall morphology. (***Circulation.*** **1994;89: 1615-1623.)**

Key Words • acetylcholine • endothelium-derived factors • atherosclerosis • transplantation • ultrasonics

Accelerated transplant atherosclerosis continues to be one of the primary problems limiting long-term survival of heart transplant patients.[1] This process is characterized histologically by diffuse myointimal proliferation of both large and small vessels.[2] Recent imaging studies using intravascular ultrasound have provided in vivo assessment of the development of intimal thickening of epicardial vessels.[3] Functional studies using acetylcholine have documented endothelial dysfunction of both large and small coronary vessels.[4-6] Experimental studies suggest that endothelial dysfunction after transplantation is due to an endothelial immune-mediated injury, which, based on the response-to-injury hypothesis, may be involved in the development of intimal proliferation and accelerated coronary graft atherosclerosis.[1] The relation of endothelial vasodilator function to structural changes detected by intravascular ultrasound appears to be complex and possibly time dependent.[7]

Reduced endothelium-dependent relaxation in response to acetylcholine is an indicator of endothelial dysfunction and has been attributed to reduced synthesis or accelerated degradation of endothelium-derived nitric oxide. Endothelial dysfunction of hypercholesterolemia affects both conduit and resistance vessels.[8-10] This abnormality can be partly corrected by the acute intravenous administration of L-arginine, the precursor of nitric oxide.[11-14]

The endothelial dysfunction that occurs in the coronary vasculature after cardiac transplantation is similar to that observed in patients with hypercholesterolemia. In both conditions it is a diffuse process that precedes angiographically visible atherosclerotic lesions and is manifest by reduced vasodilation to acetylcholine. We hypothesized that endothelial dysfunction of epicardial coronary arteries at an early stage of coronary allograft atherosclerosis might be reversed by L-arginine. To this end, the present study was designed to examine (1) whether endothelial dysfunction in epicardial coronary arteries and/or coronary microvasculature in cardiac transplant patients could be reversed by L-arginine and (2) whether baseline endothelial dysfunction and/or reversibility of endothelial dysfunction with L-arginine is correlated with the degree of intimal hyperplasia as assessed by intravascular ultrasound.

Methods

Patient Population

All cardiac transplant recipients scheduled for elective annual coronary angiography at Stanford University hospital were screened for possible participation in the study. Because of the increased angiographic contrast load associated with

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TABLE 1. Clinical Characteristics

Patient	Vessel	Age, y/Sex	Time	Rej	N Rej	Chol	LVH	WMA	CSA-L	CSA	Aza	Pred	Med	Htn	Cath
1	LCx	42 /F	10	0	. . .	209	N	N	104	150	100	8	B,Ca,F,A	Y	Mild
2	LCx	53 /F	9	0	4	258	N	N	43	150	0	10		N	Focal
3	LAD	64 /F	5	0	2	210	N	N	83	350	225	0		N	NL
4	LAD	39/M	12	0	3	208	. . .	. . .	225	400	100	10	H,B,C	Y	NL
5	LCx	24/M	10	0	7	135	N	N	243	300	200	12	A,F,P	Y	NL
6	LAD	56/M	13	0	2	183	. . .	. . .	. . .	0	50	10	Ca	Y	NL
7	LAD	44/M	1	1	6	277	N	N	138	400	25	25	A,Ca,C	Y	NL
8	LAD	60/M	2	2	. . .	206	N	Y	69	250	175	8	Ca,C	Y	NL
9	LAD	30/M	2	0	2	211	N	N	117	250	175	6	Ca,C	N	NL
10	LAD	51/M	1	0	5	193	N	N	177	700	25	16	Ca	N	NL
11	LAD	51/M	2	0	0	249	N	N	85	300	175	8	B	Y	NL
12	LAD	49/M	3	0	5	205	. . .	. . .	62	300	175	10	Ca	. . .	NL
13	LAD	52/M	8	0	2	250	. . .	. . .	70	340	50	15	A	N	Mild
14	LAD	43/M	2	0	. . .	280	N	Y	28	100	25	15	C,F	Y	NL
15	LAD	49/M	2	2	2	190	. . .	. . .	160	350	50	10	A,H,C,N	N	NL
16	LAD	49/M	4	1	. . .	146	Y	Y	. . .	450	75	4	A	N	Mild
17	LAD	45/M	4	0	1	255	. . .	. . .	45	200	225	8	Ca,H,A	Y	Mild
18	LAD	23/M	1	0	. . .	153	N	N	65	350	200	10	Ca	N	Focal

Time indicates years since transplantation (ie, 1 denotes first-year follow-up); Rej, histological grading of rejection at the time of study; N Rej, number of previous rejections; Chol, serum cholesterol level in mg/dL; LVH, echocardiographic evidence of left ventricular hypertrophy; WMA, echocardiographic evidence of left ventricular wall motion abnormality; CSA-L, cyclosporine serum level; CSA, daily cyclosporine dose; Aza, daily dose of azathioprine; Pred, daily dose of prednisone; Med, daily medication (withdrawn before study); Htn, arterial hypertension; Cath, angiographic evidence of graft coronary artery disease; LCx, left circumflex artery; LAD, left anterior descending coronary artery; N, no; Y, yes; B, β-blocker; Ca, calcium antagonist; F, furosemide; A, angiotensin-converting enzyme inhibitor; H, hydrochlorothiazide; C, clonidine; P, prazosine; N, nifedipine; and NL, no lesion.

this study and the invasive nature of the procedures, the following selection criteria were used: (1) serum creatinine <2.0 mg%, (2) no prior evidence of obstructive (transplant) coronary artery disease (ie, <30% luminal narrowing), and (3) clinically stable condition without evidence of recent rejection, infection, or other acute illness. Left ventricular hypertrophy was assessed as previously described.[15,16] The study protocol was approved by the Stanford University Committee on Human Subjects in Medical Research. All patients gave written informed consent before entry into the study. Eighteen patients who had cardiac transplantation 1 to 13 years previously were studied during their scheduled annual catheterization. The clinical characteristics of these patients are outlined in Table 1.

Doppler Guide Wire, Velocimeter, and Calculation of Blood Flow

Flow velocity measurements were made with a 0.014- or 0.018-in Doppler guide wire containing at its tip a 12-MHz pulsed Doppler ultrasound velocimeter (FlowMAP, Cardiometrics, Inc) interfaced with a real-time spectral analysis system. The technical features of this system have recently been reported in detail.[17] For the purpose of this study, the tip of the Doppler guide wire was positioned in the midportion of the left anterior descending coronary artery (LAD) (n=15) or circumflex artery (n=3) approximately 1 cm distal to the tip of a 3.6F infusion catheter. The position of the Doppler guide wire was carefully chosen to obtain a stable velocity signal and was cineangiographically documented with each injection of contrast. Doppler velocity was recorded continuously throughout the study protocol. For measurement of coronary flow, average peak velocity was multiplied by the quantified cross-sectional area of the vessel segment of interest, yielding flow in milliliters per minute as validated recently.[17,18] To avoid underestimation of true microvascular flow during epicardial coronary artery constriction in response to acetylcholine, the coronary flow was calculated only when the cross-sectional area reduction of the vessel did not exceed 50% in the most constricted epicardial segment as determined by quantitative coronary angiography[19] (one case only).

Study Design

Vasoactive medications were discontinued at least 12 hours and usually 18 to 24 hours before the study. After diagnostic angiography revealed no visually apparent coronary stenoses, an 8F guiding catheter was used to cannulate the left main coronary artery, and an additional 8000 to 10 000 U heparin was given intravenously. A 3.6F infusion catheter was then advanced over a 0.014- or 0.018-in Doppler flow velocity guide wire (Cardiometrics) into a nonbranching segment of the mid LAD or mid circumflex for infusion of acetylcholine. Acetylcholine (Miochol 10 mg/mL, Cooper Vision) was prepared with serial dilutions of normal saline to achieve infused concentrations of 10^{-6}, 10^{-5}, and 10^{-4} mol/L. This range of doses is comparable to that used in several prior published studies.[4,5,14] Based on an assumed basal blood flow of approximately 80 to 100 mL/min in the LAD, the coronary blood concentrations of acetylcholine in the LAD (and a large circumflex artery in three cases) were estimated to range from 10^{-8} to 10^{-6} mol/L. After baseline angiography was performed, increasing (logarithmic) concentrations of acetylcholine were serially infused over 3 minutes at a rate of 1 mL/min. Heart rate, blood pressure, and the ECG were continuously monitored throughout the drug infusion. At the end of each

infusion period, heart rate and blood pressure were recorded and angiography was performed, resulting in a transient increase in blood flow velocity that returned to baseline within 15 to 30 seconds. Six to eight milliliters of contrast medium was injected at intervals of at least 5 minutes to exclude dye-induced changes of coronary diameter and blood flow velocity.[20] Infusion of acetylcholine continued until the maximum dose (10^{-4} mol/L) was reached or until total coronary occlusion occurred. Then an intravenous infusion of L-arginine (10 $mg \cdot kg^{-1} \cdot min^{-1}$ over 15 minutes) was performed. Thereafter, the intracoronary infusion of acetylcholine 10^{-6}, 10^{-5}, and 10^{-4} mol/L was repeated. Coronary angiography was performed at the end of the L-arginine infusion and after the infusion of each concentration of acetylcholine. Finally, coronary arteriography was performed after the administration of 300 μg of intracoronary nitroglycerin. To ascertain the reproducibility of the acetylcholine-induced epicardial and blood flow responses, the identical protocol was performed in a separate group of four patients; however, 0.9% saline was infused intravenously instead of L-arginine.

Quantitative Angiography

Serial angiography in the same projections as at baseline was performed at the end of each infusion period and after the administration of nitroglycerin. A nonionic contrast agent, Omnipaque, was manually injected through the guiding catheter. Technically suitable single-plane angiograms were selected from biplane views for analysis. The mean diameter of a 5-mm segment of the infused vessel was measured 2 to 3 mm distal to the tip of the Doppler guide wire, since the Doppler transducer of this device has a range gate depth of 5 mm.[17,18] A 5-mm segment of a noninfused segment of a different coronary artery (mostly the left circumflex) was measured as a control. For determination of flow-dependent dilation, the diameter of a segment proximal to the infusion catheter (identified by radiopaque marker) was measured as reported previously.[20] These segments are exposed to changes in flow but not acetylcholine. The diameter measurements were made with an automated edge detection system previously described.[21] Previous studies have shown this system to have an intraobserver variability of <0.15 mm. Briefly, the segments of interest were centered, magnified, and digitized for subsequent computer analysis. Diameter measurements were performed in two consecutive end-diastolic frames and averaged to obtain the segmental vessel diameter at each time period. Calibration of the system was performed by measurement of the known guiding catheter diameter. Comparison to the baseline diameter was used to calculate the amount of vasodilatation or vasoconstriction.

Intravascular Ultrasound

After the intracoronary administration of nitroglycerin and coronary angiography, the Doppler guide wire and infusion catheter were replaced by a 0.014-in coronary guide wire. A 4.3 flexible 30-MHz ultrasound catheter (CVIS Inc, Sunnyvale, Calif) was advanced into the LAD (or left circumflex artery in three patients). The acquisition and analysis of data using this system have been reported recently.[3,22] For the purpose of this study, the imaging transducer was advanced to the same segment that served for evaluation of coronary vasomotor response to acetylcholine and for Doppler flow velocity recordings during the infusion of acetylcholine (2 to 3 mm distal to the tip of the Doppler guide wire). To identify this segment by intravascular ultrasound, the position of each image site was documented by injection of contrast with the imaging catheter left in place. Vessel branch points provided markers for accurate placement of the ultrasound catheter, which has a radiopaque rotating mirror. The 4.3F tip of the imaging catheter was not advanced to the segment of interest in 4 of 18 patients because of the small vessel diameter of the midportion of the LAD. All images were recorded on videotape for subsequent measurements from single-frame images as recently described.[22] The mean circumferential intimal thickness was measured, and an index of intimal thickness was calculated: (total area minus luminal area) divided by total area.[22] Intimal thickening was defined as ultrasound evidence of three vessel wall layers appearing over >75% of the lumen circumference, ie, grade 3 or 4. The intimal thickness was assigned to one of five classes, the descriptions of which were recently published,[3] with grade 0 denoting no intimal thickening and grades 1 and 2 being considered mild intimal thickening within normal limits.[3,23] Grade 3 or 4 refers to moderate to severe intimal thickening and is considered to represent abnormal intimal thickening.[3,23]

Statistical Analysis

All data are expressed as mean±SEM. For comparisons of hemodynamics and coronary artery dimensions and blood flow, one-way ANOVA for repeated measures was performed, followed by the Student-Newman-Keuls test. Statistical analysis of coronary artery dimensions and blood flow were made from both absolute values and percent changes from baseline and yielded similar P values. Regression lines were fitted by the method of least squares and calculated by the RS/1 program (Bolt, Beranek). A value of $P<.05$ was considered to indicate statistical significance.

Results

Systemic Hemodynamics

Mean arterial pressure, heart rate, and coronary diameter of the control vessel (not exposed to interventions) during the protocol are listed in Table 2. Blood pressure dropped significantly after the infusion of L-arginine, whereas heart rate and the diameter of the control vessel remained unchanged.

Epicardial Vasomotor Responses

Acetylcholine reduced the mean coronary segment diameter by −6.8±3.9%, from 2.37±0.12 to 2.2±0.14 mm, at acetylcholine doses of 10^{-4} mol/L (Fig 1). In 12 of 18 patients, a dose-dependent constriction of the epicardial coronary artery was observed in response to acetylcholine (mean percent change, −15.2±3.9%; range, −2% to −35%). Acetylcholine-induced vasodilation occurred in the remaining 6 patients (mean percent change, +10±2.7%; range, +2% to 20%). L-Arginine infusion did not affect coronary diameters (L-arginine, 2.40±0.11 versus 2.37±0.12 mm at baseline). However, after L-arginine infusion, acetylcholine-induced vasoconstriction was attenuated and averaged −2.8±3.6% at 10^{-4} mol/L ($P<.01$ versus acetylcholine 10^{-4} before infusion of L-arginine) (Fig 1). The effect of L-arginine was more prominent in patients with acetylcholine-induced coronary vasoconstriction at baseline (pre–L-arginine, −15.2±3.9% versus post–L-arginine, −7.5±2%; $P<.001$), whereas in the subgroup with a dilator response to acetylcholine, the effect of L-arginine was marginal and not statistically significant (pre–L-arginine, +10±2.7% versus post–L-arginine, +12.9±2.3%).

Flow-dependent dilation and intravascular ultrasound imaging of the proximal segment of the vessel under study (exposed to flow but not to acetylcholine) could be assessed in 12 patients, whereas in the remaining 6 patients, overlap of septal or diagonal branches precluded the assessment of the proximal segment of the LAD or circumflex artery. Flow-dependent dilation before L-arginine averaged 7.0±1.3% at acetylcholine

TABLE 2. Blood Pressure, Heart Rate, and Control Vessel Diameter Before and After Infusion of L-Arginine

	Blood Pressure, mm Hg	Heart Rate, bpm	Control Vessel Diameter,† mm
Baseline	112.5±6.8	83.3±3.1	2.59±0.14
Acetylcholine 10^{-6} mol/L	115.8±5.3	82.5±2.7	2.50±0.15
Acetylcholine 10^{-5} mol/L	114.6±4.7	82.5±2.7	2.62±0.14
Acetylcholine 10^{-4} mol/L	112.4±5.1	84.1±2.8	2.54±0.14
L-Arginine	96.2±4.8*	85.5±3.1	2.60±0.16
Acetylcholine 10^{-6} mol/L	98.7±5.8*	84.7±2.9	2.64±0.15
Acetylcholine 10^{-5} mol/L	101.0±5.2*	84.9±2.9	2.69±0.14
Acetylcholine 10^{-4} mol/L	105.3±5.1	85.0±2.1	2.67±0.14
Nitroglycerin	101.0±4.9	85.1±2.4	2.99±0.17

bpm indicates beats per minute. Values are mean±SEM.
*$P<.05$ as compared to corresponding acetylcholine dose pre-arginine.
†Diameter of control vessel during the protocol: circumflex artery (n=15) or left anterior descending artery (n=3).

10^{-4} mol/L (percent increase from baseline) and did not change after infusion of L-arginine (6.7±1.8%). Although the acetylcholine-induced increase in coronary blood flow was improved after L-arginine in these 12 patients (pre, +152±26% versus post; 216±26% at acetylcholine 10^{-4} mol/L), acetylcholine-induced vasoconstriction of the distal segment in these 12 patients was blunted after L-arginine (−4.7±5% versus +0.2±5%, $P<.01$ at acetylcholine 10^{-4} mol/L). Thus, the effect of L-arginine on acetylcholine-induced vasoconstriction of the distal segment cannot be attributed to greater flow-dependent dilation.

Nitroglycerin caused a dilation of 18.5±14.5%, indicating a normal dilator response to this drug. The dilator response to nitroglycerin was similar in the proximal and distal segments, suggesting a similar vasodilator capacity in proximal and distal segments.

Changes in Coronary Blood Flow

Calculated coronary blood flow showed a dose-dependent increase in response to acetylcholine from a baseline value of 35.4±4.1 to 76.2±11.6 mL/min during infusion of the highest dose (Fig 2). The acetylcholine-induced increase in coronary blood flow was significantly correlated with the acetylcholine-induced vasomotor response of the epicardial coronary artery (Fig 3), suggesting that the extents of endothelial dysfunction of epicardial vessels and microvasculature are related in graft vasculopathy. After L-arginine infusion alone, coronary blood flow did not change significantly (baseline, 35.4±4.1 mL/min; L-arginine, 39.2±5.1 mL/min). However, L-arginine significantly increased the blood flow response to acetylcholine at all three concentrations (98.2±11.1 mL/min at acetylcholine 10^{-4} mol/L) (Figs 2 and 4). The improvement of acetylcholine-induced increase in flow with L-arginine was inversely correlated with the acetylcholine-induced increase in flow before administration of L-arginine ($r=.50$; $P<.05$). Thus, the salutary effect of L-arginine on the acetylcholine-induced increase in coronary blood flow was more pronounced in patients who had

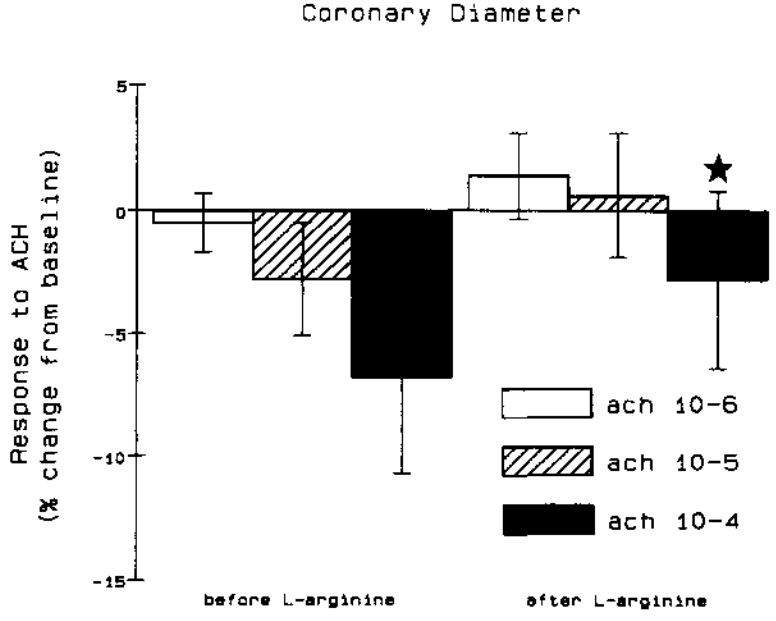

FIG 1. Bar graph showing effect of intracoronary acetylcholine (ACH, ach) on coronary diameter. The change in coronary diameter is expressed as a percentage of the baseline value (±SEM). Acetylcholine caused a dose-dependent (10^{-6}, 10^{-5}, 10^{-4} mol/L) coronary constriction; this vasoconstriction was attenuated by the administration of L-arginine. ★ indicates a significant change from corresponding value obtained before L-arginine administration ($P<.01$).

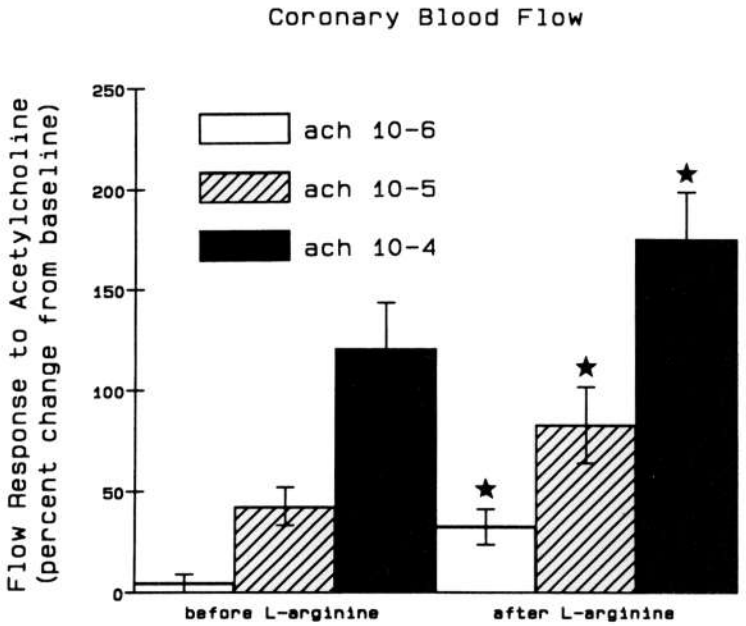

FIG 2. Bar graph showing acetylcholine (ach)-induced changes in coronary blood flow. Coronary blood flow is expressed as a percentage of the baseline value (±SEM). Acetylcholine caused a dose-dependent increase in coronary blood flow. After administration of L-arginine, the acetylcholine-induced increase in coronary blood flow was enhanced at all three concentrations of acetylcholine (10^{-6}, 10^{-5}, 10^{-4} mol/L). ★ indicates a significant change from corresponding value obtained before L-arginine administration ($P<.05$ at 10^{-6} and $P<.01$ for 10^{-5} and 10^{-4} mol/L acetylcholine).

impaired acetylcholine blood flow response before L-arginine administration.

Coronary Vessel Morphology and Response to L-Arginine

In 14 patients, vessel size allowed ultrasound imaging of the middle portion of the coronary segment that was exposed to acetylcholine. Moderate to severe intimal thickening (indicating abnormal intimal thickness, classes 3 and 4; index of intimal thickness, 0.07±0.03) was observed in 5 patients, whereas intimal thickness was within the normal range in 9 patients (class 1 or 2; index of intimal thickness, 0.48±0.05). In 5 of 9 patients with normal vessel wall morphology, the epicardial coronary artery constricted in response to the highest dose (10^{-4} mol/L) of acetylcholine (10^{-5} mol/L in 4 of 9 patients). In 4 of 5 patients with abnormal intimal thickness, acetylcholine (10^{-5} and 10^{-4} mol/L) caused coronary vasoconstriction ($P<.01$ by χ^2 test). These results indicate that acetylcholine-induced coronary vasoconstriction may precede the onset of abnormal intimal thickness detected by intravascular ultrasound in these cardiac transplant recipients.

Flow-dependent dilation was impaired in patients with abnormal intimal thickness (+3.8±0.6%) compared with patients without abnormal intimal thickening (9.2±1.7%; $P<.05$), although the acetylcholine-

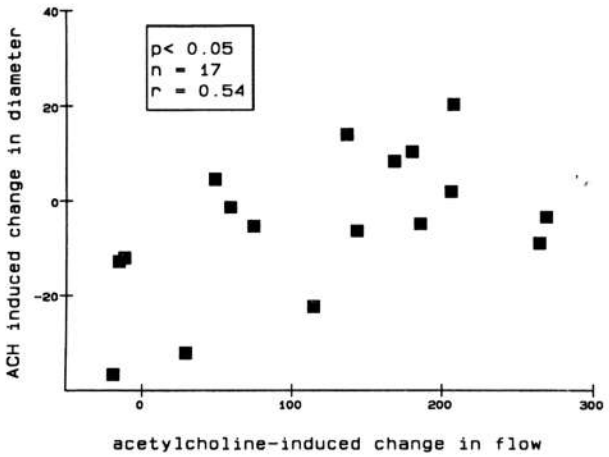

FIG 3. Scatterplot showing correlation of acetylcholine (ACH)-induced increase in coronary blood flow (percent from baseline) and acetylcholine-induced change in coronary diameter (percent change from baseline).

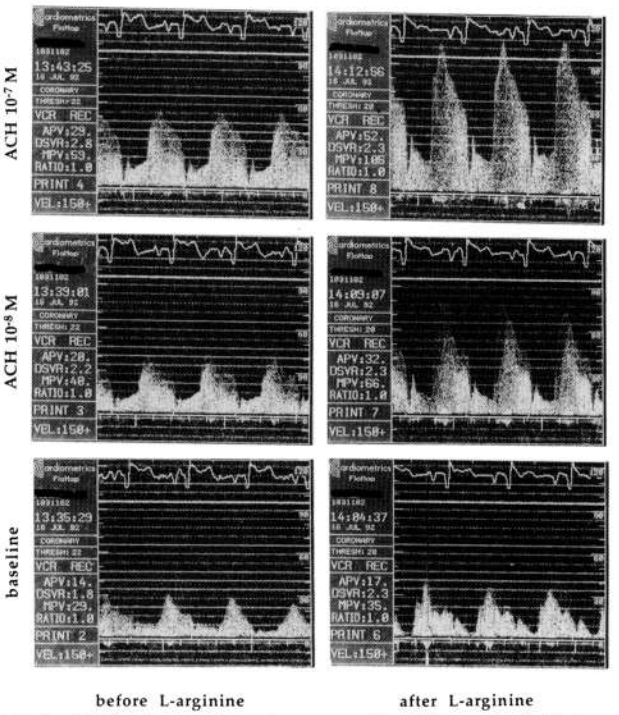

FIG 4. Original recording of coronary blood flow velocity before (left) and after (right) administration of L-arginine. After L-arginine, the acetylcholine-induced increase in coronary blood flow velocity (10^{-6}, 10^{-5}, and 10^{-4} mol/L) is substantially enhanced. Note that the scale is changed for acetylcholine 10^{-4} mol/L after L-arginine to record the increased flow velocity.

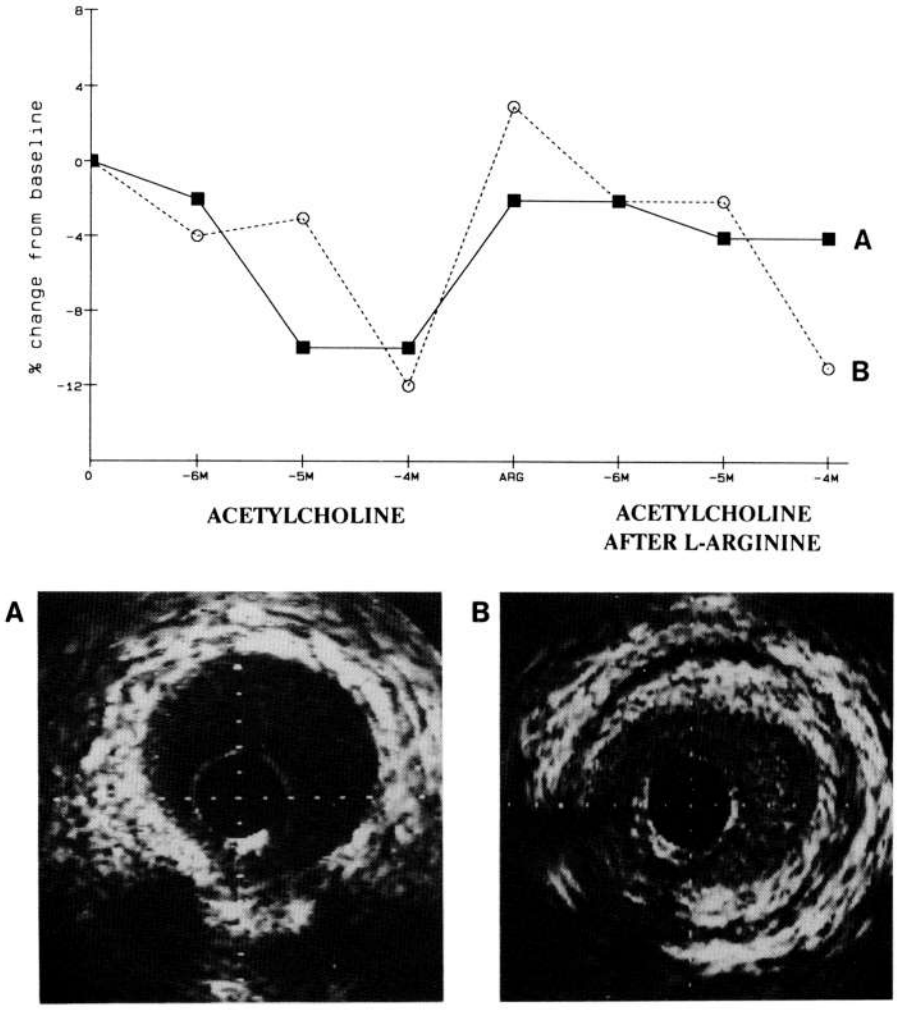

FIG 5. Graph and intravascular ultrasound images showing effect of L-arginine (ARG) on coronary diameter in a patient without intimal thickening (A) and a patient with substantial intimal thickening (B) as documented by intravascular ultrasound. The acetylcholine-induced coronary vasoconstriction at 10^{-5} and 10^{-4} mol/L is attenuated after the administration of L-arginine in the patient without intimal thickening (A). In patient B, with substantial intimal thickening, L-arginine did not affect the acetylcholine-induced coronary vasoconstriction.

induced increase in blood flow was similar in both subgroups (+137% versus 168%, NS).

In 7 of 9 patients with normal intimal thickness, the epicardial response to acetylcholine was improved after L-arginine. Only 2 of 5 patients with abnormal intimal thickness showed attenuation of the acetylcholine-induced vasoconstrictor response after L-arginine ($P<.01$ by χ^2 test compared with grade 1 or 2 patients). This suggests that a positive response to acetylcholine after L-arginine was more likely to improve the epicardial response to acetylcholine in coronary arteries without intimal thickening as identified by intravascular ultrasound (see Fig 5).

Reproducibility of Acetylcholine-Induced Coronary Responses

In four additional posttransplant patients, the epicardial responses and coronary blood flow changes were assessed before and after intravenous infusion of saline. The results, outlined in Table 3, indicate a good reproducibility of acetylcholine-induced epicardial vasomotor changes and coronary blood flow responses. There were no significant differences in the epicardial and blood flow responses to acetylcholine when corresponding data of the first and second challenges with acetylcholine were compared. It should be noted that the flow velocity signal was remarkably stable, probably because of the stabilizing effect of the proximal infusion catheter on the flow wire.

Discussion

The results of the present study demonstrate that the coronary vessels of cardiac transplant patients exhibit a diffuse endothelial dysfunction that is attenuated or

TABLE 3. Reproducibility of Acetylcholine-Induced Epicardial and Coronary Blood Flow Responses

	Diameter, mm	Coronary Flow, mL/min
Baseline	2.73±0.3	40.3±7.6
Acetylcholine 10^{-6} mol/L	2.55±0.3	37.4±7.9
Acetylcholine 10^{-5} mol/L	2.40±0.4	53.1±5.3
Acetylcholine 10^{-4} mol/L	2.51±0.4	84.5±16.7
Baseline 2	2.62±0.5	33.5±7.1
Acetylcholine 10^{-6} mol/L	2.48±0.4	36.6±9.8
Acetylcholine 10^{-5} mol/L	2.35±0.4	48.2±10.8
Acetylcholine 10^{-4} mol/L	2.53±0.5	85.0±20.4

Data are mean±SEM of four patients. No significant differences in the response to acetylcholine were found between first and second dose-responses.

reversed by intravenous administration of L-arginine, the precursor of nitric oxide. The reversibility of epicardial endothelial dysfunction by L-arginine is more likely in vessels without increased intimal thickening.

Accelerated coronary atherosclerosis has emerged as a major cause of morbidity and mortality in cardiac transplant recipients. Although the mechanism of transplant coronary artery disease is still incompletely understood, endothelial dysfunction appears to occur soon after transplantation and may be involved in the development of graft atherosclerosis. The present study suggests that endothelial dysfunction can be identified before intimal thickening, which is readily detected and accurately graded by intravascular ultrasound.[3,23] The present observations extend earlier reports from this institution[5] and Fish et al[4] demonstrating paradoxical coronary vasoconstriction to acetylcholine in cardiac transplant recipients. Although endothelial dysfunction has been reported to occur in some patients before the development of angiographic lesions,[4] this earlier study did not include intravascular ultrasound, a more sensitive method for evaluating structural abnormalities. Several studies from this institution have now documented that intimal thickening precedes angiographically visible coronary artery disease in cardiac transplant recipients.[3,23] The present study delineates the relation between coronary endothelial dysfunction (of both epicardial arteries and microvasculature) and intimal thickening by use of coronary angiography, Doppler flow velocity technology, and intravascular ultrasound.

The vasodilator effect of acetylcholine in large human coronary arteries can be reversed to vasoconstriction after pretreatment with N^G-monomethyl L-arginine, an inhibitor of nitric oxide synthesis from L-arginine.[24] This observation indicates that the dilator response elicited by acetylcholine in humans is mediated by the release of nitric oxide. Conversely, acetylcholine elicits coronary vasoconstriction (by direct action on vascular smooth muscle) when the synthesis or release of nitric oxide is inhibited or impaired. Flow-dependent dilation of large vessels appears to be endothelium-mediated because of increased release of endothelium-derived relaxing factor (EDRF).[25,26] Thus, paradoxical vasoconstriction in response to acetylcholine and impaired flow-mediated vasodilation indicates that the endothelial release of nitric oxide in response to these stimuli is impaired in many cardiac transplant recipients. Notably, a vasodilator response to acetylcholine was observed predominantly in patients without excessive intimal thickening. Similarly, it appears that the degree of flow-dependent dilation was preserved in coronary arteries with near-normal vessel wall composition but attenuated in coronary arteries with abnormal intimal thickening. It should be noted that a maximal increase in flow (ie, by adenosine or papaverine) is necessary to elicit maximal flow-dependent dilation, whereas the increases in flow achieved by acetylcholine are not maximal. The present finding, however, may suggest that flow-dependent dilation at submaximal increases in flow (rather than maximal flow-dependent dilation) is attenuated in vessels with abnormal vessel wall. Conceivably, the differences between normal vessels and those with structural alterations may be more marked with maximal increases in flow. We have previously shown that there is a linear relation between the degree of increase in flow and the degree of flow-dependent dilation.[18]

Interestingly, the beneficial effect of L-arginine on epicardial vasomotor responses was prominent in patients whose epicardial coronary artery constricted in response to acetylcholine at baseline, whereas the (normal) acetylcholine-induced dilator response in six patients was not significantly enhanced by L-arginine. Thus, the potential beneficial effect of L-arginine appears to depend on the presence of endothelial dysfunction.

Although the reversibility of endothelial dysfunction was related to the presence of intimal thickening, the initial (prearginine) endothelial dysfunction was not always predicted by the degree of intimal thickening. In three patients with coronary endothelial dysfunction 10 to 12 years after transplantation, intimal thickness was within normal range (<300 μm).[3,23] Thus, although endothelial dysfunction may precede intimal thickening, other factors are crucial in the development of intimal thickening and graft atherosclerosis. Interestingly, preliminary data from our laboratory suggest that acetylcholine can cause paradoxical vasoconstriction early after cardiac transplantation, whereas the identical segment demonstrated completely preserved flow-dependent dilation (unpublished observations). Moreover, the dilator response to substance P, another endothelium-dependent agent, appears to be better preserved in cardiac transplant patients.[27] These observations suggest that impairment of muscarinic vasorelaxation may emerge early and selectively in posttransplantation endothelial dysfunction. Notably, such a selective impairment of muscarinic vasorelaxation has previously been shown in isolated atherosclerotic human coronary arteries.[28]

Normal humans exhibit acetylcholine-induced dose-dependent dilation of the coronary microvasculature,[6,14,17,29,30] an effect that is likely to be due to EDRF-mediated vasodilation of small coronary arteries.[29] In individuals with a normal coronary flow reserve to papaverine or adenosine,[14,30,31] intracoronary infusion of acetylcholine, administered in doses up to 10^{-4} mol/L, increases coronary blood flow by 150% to 250%. In the present study, the acetylcholine-induced increase in coronary blood flow averaged 120%; however, in seven cardiac transplant recipients, the maximal increase in flow was <75%. We did not assess coronary flow reserve (ie, using papaverine or adenosine) in these patients and therefore cannot exclude the possibility that the impaired blood flow response to acetylcholine was, in part, related to an inability of the arteriolar smooth muscle to dilate. However, previous studies have shown that coronary flow reserve is normal in cardiac transplant recipients in the absence of left ventricular hypertrophy or wall motion abnormalities.[32] Thus, the impaired relaxation of the coronary microvasculature in response to acetylcholine is likely to be due to endothelial dysfunction of the coronary microvasculature, particularly in those patients with severely depressed blood flow response to acetylcholine. The beneficial effect of L-arginine was observed predominantly in patients with severely depressed blood flow response to acetylcholine, supporting the notion that endothelial dysfunction of the coronary microcirculation was present in these patients and was improved by administra-

tion of the precursor of nitric oxide (or EDRF), L-arginine. Interestingly, endothelial dysfunction of epicardial coronary arteries and that of the corresponding microvasculature were correlated, supporting the view that a common injury may play an important role in the pathogenesis of coronary endothelial dysfunction in both large and small coronary vessels of cardiac transplant recipients.

It is now well established that nitric oxide is synthesized from L-arginine and accounts for the biological activity of EDRF.[33,34] In the present study, L-arginine was used to enhance the synthesis of EDRF and thereby influence one important function of the endothelium. The beneficial effect of L-arginine on endothelial function in the coronary microcirculation is consistent with our previous observations in patients with hypercholesterolemia.[14] In that study, L-arginine corrected the endothelial dysfunction of the coronary microcirculation in patients with hypercholesterolemia but did not affect the acetylcholine-induced blood flow response in normal individuals. Similarly, the beneficial effect of L-arginine in cardiac transplant recipients was inversely related to acetylcholine-induced blood flow response before L-arginine.

The mechanism of impaired endothelium-dependent dilation remains controversial. In addition to altered membrane receptor coupling mechanisms affecting the release of EDRF, a reduced uptake of the precursor, a reduced synthesis of EDRF, or impaired diffusion or augmented destruction of EDRF in the vessel wall could be involved. Based on the K_d of nitric oxide synthase for L-arginine (the enzyme catalyzing the formation of nitric oxide), L-arginine should not be a rate-limiting substrate. However, the biochemical characteristics of the nitric oxide synthase have been assessed in cultured endothelial cells. There is evidence that the intracellular availability of L-arginine may become a rate-limiting factor for the production of nitric oxide in endothelium exposed to oxidized low-density lipoprotein.[35] The improvement of endothelium-dependent dilation within the human coronary circulation after administration of L-arginine would be consistent with the hypothesis that by providing the substrate for synthesis of EDRF, enhanced formation of EDRF restores endothelium-dependent dilation. Notably, beneficial effects of L-arginine have been reported for several cardiovascular disorders associated with endothelial dysfunction, such as hypercholesterolemia, reperfusion, and hypertension.[11,14,36] Experimental studies have documented that L-arginine stereospecifically improves the endothelium-dependent relaxation (ie, D-arginine was ineffective).[12,37]

Taken together, these observations suggest that endothelial dysfunction as defined by the response to acetylcholine is associated with a reversible reduction of EDRF availability that can be overcome by L-arginine supplementation. If so, supplementation of L-arginine could represent a novel therapeutic strategy to maintain normal endothelial function. Moreover, L-arginine might prevent the development or progression of atherosclerosis. In support of this, Cooke et al[38] found that long-term dietary L-arginine treatment improved endothelial function in hypercholesterolemic rabbits and, more importantly, attenuated the development of atherosclerosis. Posttransplant endothelial dysfunction could serve as an ideal model to test the preventive effect of L-arginine in humans, since, in contrast to hypercholesterolemia, L-arginine could be instituted early after endothelial injury, and annual coronary angiography would allow the assessment of endothelial function and intimal thickening during follow-up.

Some limitations of the present study should be noted. First, infusion of L-arginine caused a transient but significant fall in arterial blood pressure, which might have affected the vasomotor responses to acetylcholine. However, McGinn et al[39] have shown that changes in heart rate but not blood pressure alter coronary flow reserve in cardiac transplant recipients. Heart rate remained constant throughout the study, consistent with denervation of the transplant heart, limiting the potential of changes in blood pressure to interfere with coronary flow through reflex control mechanisms. Moreover, arterial blood pressures during infusion of acetylcholine 10^{-4} mol/L was similar before and after L-arginine; yet the effect of L-arginine was most prominent at this dose. Second, the beneficial effect of L-arginine on acetylcholine-induced epicardial vasoconstriction could be, in part, related to increased blood flow responses to acetylcholine after L-arginine. However, flow-dependent dilation in the proximal segments (whose vessel wall morphology and dilator responses to nitroglycerin are similar to those of the distal segments) was not affected after L-arginine. Thus, it appears unlikely that the beneficial effect of L-arginine on acetylcholine-induced epicardial vasoconstriction can be attributed to a greater flow-dependent dilation. It should be noted that the effects of L-arginine on acetylcholine-induced epicardial responses were small, and therefore, independent confirmation of the present observation in a larger patient population is warranted.

In summary, the present study provides evidence that endothelial dysfunction can precede intimal thickening in cardiac transplant recipients. L-arginine, the precursor of EDRF, improves endothelial function of epicardial coronary arteries and the coronary microvasculature in cardiac transplant recipients. These findings substantially extend the earlier findings in hypercholesterolemic patients by demonstrating that L-arginine is effective in cardiac graft vasculopathy. Importantly, by combined assessment of vessel wall morphology with intravascular ultrasound and vascular responses to acetylcholine by quantitative angiography and Doppler guide wire, this study delineates the relation between L-arginine effects and vessel wall morphology.

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