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Coronary Endothelial Dysfunction in Patients With Acute-Onset Idiopathic Dilated Cardiomyopathy

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Objectives. **This study sought to determine whether coronary endothelial dysfunction exists in patients with acute-onset idiopathic dilated cardiomyopathy (DCM) and to explore its relation to recovery of left ventricular systolic function in this patient population.**

Background. **Coronary endothelial dysfunction exists in chronic DCM, but its importance in the development and progression of ventricular dysfunction is not known. To address this issue we studied coronary endothelial function in patients with idiopathic DCM <6 months in duration and explored the relation between coronary endothelial function and subsequent changes in left ventricular ejection fraction (LVEF).**

Methods. **Ten patients with acute-onset idiopathic DCM (duration of heart failure symptoms 2.0 ± 0.4 months [mean ± SEM]) and 11 control patients with normal left ventricular function underwent assessment of coronary endothelial function during intracoronary administration of the endothelium-dependent vasodilator acetylcholine and the endothelium-independent vasodilator adenosine. Coronary cross-sectional area (CSA) was determined by quantitative coronary angiography and coronary blood flow (CBF) by the product of coronary CSA and CBF velocity measured by an intracoronary Doppler catheter. Patients with DCM underwent assessment of left ventricular function before and several months after the study.**

Results. **Acetylcholine infusion produced no change in coronary CSA in control patients but significant epicardial constriction in patients with DCM (−36 ± 11%, $p < 0.01$). These changes were associated with increases in CBF in control patients (+118 ± 49%, $p < 0.01$) but no change in patients with DCM. Infusion of adenosine produced increases in coronary caliber and blood flow in both groups. Follow-up assessment of left ventricular function was obtained in nine patients with DCM 7.0 ± 1.7 months after initial study, at which time LVEF had improved by ≥0.10 in four patients. Multiple linear regression revealed a positive correlation between both the coronary CSA ($r^2 = 0.57$, $p < 0.05$) and CBF ($r^2 = 0.68$, $p < 0.01$) response to acetylcholine and the subsequent improvement in LVEF.**

Conclusions. **Coronary endothelial dysfunction exists at both the microvascular and the epicardial level in patients with acute-onset idiopathic DCM. The preservation of coronary endothelial function in this population is associated with subsequent improvement in left ventricular function.**

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The etiology of the ventricular dilation and dysfunction that occur in idiopathic dilated cardiomyopathy (DCM) is unknown. Although some studies have observed inflammation in response to a viral or autoimmune insult (1,2) in patients with DCM, frequently no evidence of infection, autoantibodies or inflammation can be demonstrated (3,4). Even when active inflammation can be found, it is often patchy (5) and therefore does not readily explain the global nature of the myocardial dysfunction seen in these patients. It has been hypothesized that diffuse subendocardial ischemia due to altered coronary physiology may contribute to the global cardiac dysfunction seen in DCM (6) and that more focal coronary involvement may account for the regional ventricular wall motion abnormalities that are sometimes observed (7).

Abnormal responses to vasodilating stimuli have been demonstrated in patients (8–11) with, and in animal models (12,13) of, DCM. Abnormalities in the vasodilator response of both the coronary (8,10,11) and the systemic (9,14) circulation have been identified. These studies have documented a failure to augment blood flow in response to a variety of stimuli known to cause arterial dilation through endothelium-dependent mechanisms. The finding that the blunted coronary responses of patients with DCM to endothelium-dependent vasodilators occur in the absence of epicardial constriction has been used to suggest that the vasomotor abnormalities in DCM are restricted to the microvasculature (8).

Previous investigations of coronary vasomotor responses in patients with DCM have studied patients with chronic DCM (11) or have not specified the duration of DCM (8–10). Therefore, it has not been possible to determine whether coronary vascular abnormalities exist early in the course of

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Abbreviations and Acronyms

CBF = coronary blood flow
CHF = congestive heart failure
CSA = cross-sectional area
DCM = dilated cardiomyopathy
LVEF = left ventricular ejection fraction
LVSWI = left ventricular stroke work index
MAP = mean arterial pressure
PCWP = pulmonary capillary wedge pressure
C1 = control solution of 5% dextrose in water (acetylcholine vehicle)
C2 = control solution of 0.9% NaCl (adenosine vehicle)

DCM. Insights into the potential role of these abnormalities in the pathogenesis and progression of DCM have consequently been elusive. To determine whether abnormal coronary physiology exists early in the course of DCM, we studied coronary vascular responses to both the endothelium-dependent vasodilator acetylcholine and the endothelium-independent vasodilator adenosine in patients with acute-onset (within the preceding 6 months) DCM. We reasoned that if abnormal coronary physiology does not exist early in the course of DCM, then it is unlikely to play a role in the pathogenesis and progression of the myopathic state. In addition, we sought to define the relation between coronary endothelial function early in the course of DCM and subsequent changes in left ventricular function.

Methods

Study patients. The study group included seven men and three women with acute-onset DCM. Patients were eligible for the study if they had symptoms of congestive heart failure (CHF), such as dyspnea, fatigue or edema for ≤6 months and were found to have a left ventricular ejection fraction (LVEF) ≤0.40. Patients were excluded from study if they had decompensated CHF preventing them from resting supine, a history of heavy ethanol abuse, severe hypertension, primary valvular disease or other known cause of DCM. The control group included six men and five women with undiagnosed chest pain and normal left ventricular chamber dimensions (left ventricular end-diastolic dimension <55 mm) and systolic function (LVEF >0.50). Seven of the 11 control patients underwent treadmill exercise testing with thallium imaging within 8 weeks of catheterization. No test showed electrocardiographic evidence of ischemia. Six of seven patients had no defects on thallium imaging, and the remaining patient had a mild superior septal reversible defect. All subjects underwent diagnostic cardiac catheterization before the study and were found to have no significant epicardial atherosclerotic disease. The study protocol was approved by the subcommittee on human studies at Massachusetts General Hospital. Written informed consent was obtained from all patients.

Cardiac catheterization. After an overnight fast, patients received premedication with 5 mg of diazepam and 25 to 50 mg of diphenhydramine and were brought to the catheterization laboratory. All vasoactive medications, including alpha- and beta-adrenergic blocking agents, calcium channel blocking agents, angiotensin converting-enzyme inhibitors, digoxin and nitrates were withheld for 12 to 24 h before the procedure.

Internal jugular venous and femoral artery access was obtained and systemic anticoagulation achieved with 3,000 to 5,000 U of intravenous heparin. Right and left heart catheterization with coronary angiography and, in some cases (six patients with DCM, eight control patients), left ventriculography were performed. Nonionic contrast media (Omnipaque, Nycomed) was used in all cases. After diagnostic catheterization, additional intravenous heparin was given to reach a total of 10,000 U. An 8F guiding catheter was placed into the ostium of the left coronary artery. A 3F catheter with a 20-MHz pulsed Doppler crystal tip (Millar Instruments) was placed over a 0.010-in. high torque floppy guide wire and into the guiding catheter. The use of this catheter for the measurement of relative changes in coronary blood flow (CBF) velocity has been previously described (15,16). The Doppler catheter was connected to a zero-cross velocimeter (Millar Instruments) from which flow velocity signals were obtained. Under fluoroscopic guidance the guide wire was advanced into the middle portion of the left anterior descending or, in one control patient, into the nondominant left circumflex coronary artery. The Doppler-tipped catheter was then advanced over the wire into the proximal portion of the artery, and the wire was removed. The quality of the Doppler signal was checked, and the image intensifier position was adjusted to provide the optimal view of the vessel, with priority given to the portion within 5 mm of the Doppler tip. Thereafter, the imaging geometry and patient were kept stationary to ensure reproducibility of the angiographic view. The Doppler range gate was then adjusted to optimize the flow velocity signal and was subsequently held constant throughout the protocol. Coronary angiography was performed through the guiding catheter using a power injector (Medrad) set to deliver contrast medium at a constant volume and rate (8 to 9 ml at 5 to 7 ml/s).

Experimental protocol. After a control period, the following agents were administered into the coronary artery at 0.8 ml/min through the Doppler catheter for 3 min using an infusion pump (Baxter Healthcare): 1) a control solution (C1) of 5% dextrose in water (the acetylcholine vehicle); 2) three successive doses of acetylcholine, to yield estimated coronary concentrations of 10^{-8}, 10^{-7} and 10^{-6} mol/liter (assuming coronary artery blood flow of 80 ml/min [17]); 3) a control solution (C2) of 0.9% NaCl (the adenosine vehicle); and 4) adenosine to achieve an estimated coronary concentration of 10^{-4} mol/liter, a dose previously shown to produce maximal coronary vasodilation (18). Heart rate, mean arterial pressure (MAP) and mean CBF velocity were monitored during all infusions. After 3 min of each infusion, and before terminating the infusion, these hemodynamic variables were recorded, and coronary angiography was performed. Drug infusion was stopped and the study protocol terminated in the event of the patient developing prolonged chest pain, electrocardiographic

Table 1. Clinical Characteristics of Patients With Acute-Onset Dilated Cardiomyopathy

Pt. No./ Gender	Age (yr)	Duration of Sx (mo)	NYHA Functional Class	LVEF	LVEDD (mm)	Biopsy Result
1/F	63	0.75	2	0.25	55	H
2/F	35	2	2	0.30	52	Nl
3/M	51	0.5	2	0.18	99	Fib, H
4/M	18	3	2	0.27	74	Fib, H
5/M	63	5	2	0.40	59	Fib
6/M	30	2	2	0.25	74	Fib, H
7/M	44	1	2	0.12	63	H
8/M	35	0.75	2	0.16	60	H
9/M	71	3	3	0.30	67	Fib, H
10/F	57	2	3	0.22	56	Fib, H
Mean ± SEM	47 ± 5	2.0 ± 0.4	2.2 ± 0.1	0.25 ± 0.03	66 ± 4	

F = female; Fib = fibrosis; H = myocyte hypertrophy; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; M = male; Nl = normal biopsy; NYHA = New York Heart Association; Pt = patient; Sx = heart failure symptoms.

evidence of ischemia or severe epicardial coronary constriction.

Quantitative coronary angiography. Coronary angiograms were analyzed using a CMS workstation (Medis, Leiden, The Netherlands), the use and validation of which have been described previously (19,20). For each condition, the first end-diastolic frame in which the study artery was fully opacified was chosen and digitized in a matrix size of 512 × 512 pixels with 8-bit brightness resolution. Images were calibrated to the guiding catheter. A minimal cost contour detection algorithm was used, and edge detection was modified by data from the modulation transfer function of the system. The mean coronary artery diameter was measured over a 1-mm length surrounding the Doppler range. Coronary artery cross-sectional area (CSA) was then calculated, assuming a cylindrical artery, by the equation $CSA = (D^2)\pi/4$, where D is the quantitative diameter of the study vessel. CBF was then calculated as the product of mean CBF velocity and coronary CSA. Relative changes in CBF and CSA are reported as the percent change compared with the preceding control infusion.

Hemodynamic measurements. Initial LVEF was determined by echocardiography (n = 15 [9 patients with DCM, 6 control patients]) within the 3 weeks before the study for patients with DCM and within 12 weeks of the study for control patients or by contrast angiography (n = 6 [1 patient with DCM, 5 control patients]) at the time of study. Follow-up LVEF was determined by echocardiography (n = 8) or radionuclide ventriculography (n = 1). Left ventricular stroke work index (LVSWI) was calculated as (MAP − PCWP) × SVI × 0.0136, where PCWP is pulmonary capillary wedge pressure, and SVI is stroke volume index, calculated as the quotient of cardiac index and heart rate. The rate–pressure product was calculated as the product of MAP and heart rate.

Endomyocardial biopsy. After completion of the drug infusion protocol, patients with DCM, at the discretion of the referring physician, underwent right ventricular (n = 4), left ventricular (n = 1) or biventricular (n = 5) endomyocardial biopsy. Right ventricular biopsies were performed through the right internal jugular or femoral vein using a bioptome (Cordis) advanced through a sheath to the right ventricular apical septum as guided by orthogonal fluoroscopic views. Left ventricular biopsies were performed through the femoral artery using a bioptome advanced through a long sheath to the left ventricular inferior wall. Four biopsy samples were taken from each ventricle, with an effort made to vary the sampling site within the ventricle. Specimens were fixed in buffered 10% formalin. Paraffin-embedded samples were sectioned and stained with hematoxylin-eosin, Masson's trichrome and Congo red and were examined by light microscopy. Specimens were categorized according to previously published criteria (21), and myocyte hypertrophy and fibrosis were graded by semiquantitative means.

Statistical analysis. Results are expressed as mean value ± SEM. Clinical and hemodynamic comparisons between groups were made using the unpaired Student *t* test or chi-square test, as appropriate. Comparisons between conditions were made using two-way analysis of variance, followed by the paired Student *t* test corrected for repeated measures (using the Fisher least significant difference test). These analyses were performed after the interaction term in the two-way analysis of variance was found to not be significant ($p > 0.10$). Changes in coronary CSA and CBF are reported as significant if both the actual values of CSA and CBF and the relative changes in these variables reached significance. Multiple linear regression was used to examine the relation between clinical and hemodynamic variables (age, duration of CHF symptoms, serum sodium, initial LVEF, heart rate, MAP, PCWP, LVSWI and coronary CSA and CBF responses to acetylcholine and adenosine) and follow-up LVEF. Significance was considered to be at the $p < 0.05$ level.

Results

Clinical characteristics. Table 1 summarizes selected clinical and laboratory characteristics of the patients with acute-onset DCM. There were no differences in age (47 ± 5 [range

Table 2. Baseline Hemodynamic Variables

	DCM Group (n = 10)	Control Group (n = 11)	p Value
LVEF	0.25 ± 0.03	0.66 ± 0.02	< 0.01
HR (beats/min)	90 ± 6	68 ± 4	< 0.01
MAP (mm Hg)	86 ± 3	106 ± 3	< 0.01
RPP (mm Hg/min)	7,783 ± 700	7,312 ± 517	NS
PCWP (mm Hg)	13 ± 2	7 ± 1	< 0.01
LVSWI (g·m/m^2)	31 ± 4	52 ± 5	< 0.01

Data presented are mean value ± SEM. DCM = dilated cardiomyopathy; HR = heart rate; LVEF = left ventricular ejection fraction; LVSWI = left ventricular stroke index; MAP = mean arterial pressure; PCWP = pulmonary capillary wedge pressure; RPP = rate–pressure product.

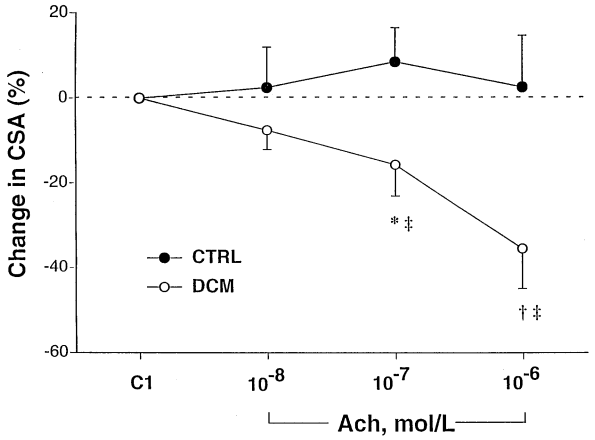


Figure 1. Epicardial CSA response to graded intracoronary doses of acetylcholine (Ach) and a 5% dextrose control infusion (C1) in patients with acute-onset DCM and in control patients (CTRL). Acetylcholine infusion produced no change in CSA in control patients but a significant epicardial constriction in patients with DCM. *$p < 0.05$, †$p < 0.01$ versus C1. ‡$p < 0.05$ versus control patients.

18 to 71] vs. 58 ± 3 [range 36 to 75] years) or gender distribution (three women, seven men vs. five women, six men, p = NS for both) between the DCM and control groups. The mean duration of CHF symptoms in patients with DCM was 2.0 ± 0.4 months (range 0.5 to 5). All patients with DCM were in New York Heart Association class II or III at the time of study. The groups did not differ in the prevalence of the following risk factors for atherosclerotic disease: smoking (5 [50%] of 10 patients with DCM vs. 8 [73%] of 11 control patients); hyperlipidemia (total serum cholesterol ≥200 mg/dl) (4 [40%] of 10 patients with DCM vs. 7 [64%] of 11 control patients); diabetes mellitus (0 [0%] of 10 patients with DCM vs. 3 [27%] of 11 control patients). There was a greater prevalence of a history of hypertension in the control group (1 [10%] of 10 patients with DCM vs. 9 [82%] of 11 control patients, $p < 0.05$).

Hemodynamic measurements. All patients were in sinus rhythm at the time of the study, with the exception of three patients with DCM (Patients 5, 8, 9) who were in atrial fibrillation with a controlled ventricular response. Baseline hemodynamic variables for the two groups are shown in Table 2. The DCM group had a lower LVEF, MAP and LVSWI and a higher heart rate and PCWP than the control group. The rate-pressure product did not differ between the groups. During acetylcholine infusion, the rate–pressure product increased by ~10% in both groups: peak rate–pressure product 8,694 ± 902 mm Hg/min with 10^{-6} mol/liter acetylcholine in the DCM group; peak rate–pressure product 8,016 ± 458 mm Hg/min with 10^{-7} mol/liter acetylcholine in the control group (both $p < 0.05$ vs. C1). At no dose of acetylcholine or adenosine did the rate–pressure product differ between groups, and at each point in the protocol MAP remained lower and heart rate higher in patients with DCM than in control patients.

Coronary responses. The drug protocol was completed in 19 of 21 patients (10 patients with DCM, 9 control patients). In the two control patients who did not complete the protocol, the highest concentration of acetylcholine was not given because of apparent severe epicardial constriction during infusion of the 10^{-7} mol/liter acetylcholine dose in one patient and prolonged chest pain without epicardial constriction or electrocardiographic evidence of ischemia in the remaining patient. Coronary CSA and CBF at baseline did not differ in the two groups (CSA 6.4 ± 1.0 vs. 4.2 ± 1.0 mm^2, CBF 29.3 ± 5.6 vs. 23.3 ± 4.4 ml/min; DCM group vs. control group, p = NS for both).

Infusion of acetylcholine produced no change in coronary CSA in the control group but did produce epicardial constriction in patients with DCM (Fig. 1) In the DCM group, CSA did not change with 10^{-8} mol/liter acetylcholine (−6 ± 5% vs. C1, p = NS) but decreased progressively with each of the higher doses (10^{-7} mol/liter acetylcholine: −17 ± 8% vs. C1, $p < 0.05$; 10^{-6} mol/liter acetylcholine: −36 ± 11% vs. C1, $p < 0.01$; both $p < 0.05$, DCM group vs. control group). Overall, 8 (80%) of the 10 patients with DCM had a decrease in CSA with 10^{-7} mol/liter acetylcholine compared with 4 (36%) of the 11 control patients (p = 0.08, DCM group vs. control group).

Infusion of acetylcholine produced an increase in CBF in control patients but no change in patients with DCM (Fig. 2). CBF increased in the control group with both 10^{-7} and 10^{-6} mol/liter acetylcholine (10^{-7} mol/liter acetylcholine: +53 ± 24% vs. C1; 10^{-6} mol/liter acetylcholine: +118 ± 49% vs. C1; both $p < 0.01$ vs. C1). In contrast, there was no change in CBF in the DCM group with any dose of acetylcholine. The percent change in CBF was higher in control patients than in patients with DCM with 10^{-6} mol/liter acetylcholine (+118 ± 49% vs. +1 ± 18%, $p < 0.05$).

Infusion of 10^{-4} mol/liter adenosine produced a similar increase in CSA and CBF in both groups (Fig. 3) (CSA: +37 ± 10% for DCM group, +20 ± 8% for control group, both $p < 0.01$ vs. C2; CBF: +234 ± 53% for DCM group, +247 ± 64% for control groups, both $p < 0.01$ vs. C2); (DCM vs. control, p = NS for both CSA and CBF changes).

Endomyocardial biopsy. The results of endomyocardial biopsy are shown in Table 1. No patient was found to have myocarditis or borderline myocarditis. However, 8 (80%) of 10 patients were found to have myocyte hypertrophy, 6 (60%) of

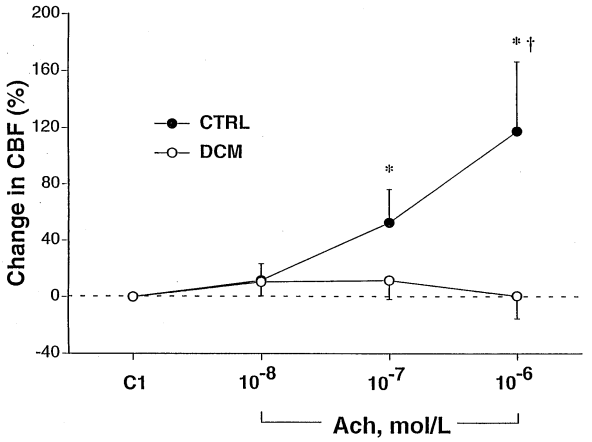


Figure 2. CBF response to graded intracoronary doses of acetylcholine (Ach) and a 5% dextrose control infusion (C1) in patients with acute-onset DCM and in control patients (CTRL). Acetylcholine infusion produced an increase in CBF in control patients but no change in patients with DCM. *p < 0.01 versus C1. †p < 0.05 versus patients with DCM.

Table 3. Multiple Linear Regression Analysis of Clinical and Hemodynamic Variables and Follow-Up Left Ventricular Ejection Fraction

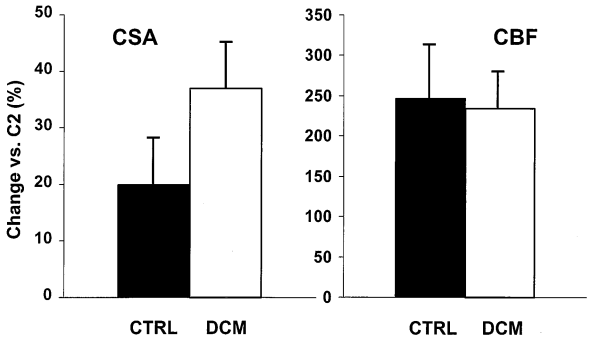
Variable	r^2 Coeff	F Value	p Value
Age (yr)	0.09	0.68	0.44
Duration of Sx (mo)	0.08	0.64	0.45
Serum sodium (mEq/liter)	0.02	0.12	0.74
Initial LVEF	0.02	0.12	0.74
HR (beats/min)	0.07	0.50	0.50
MAP (mm Hg)	0.05	0.36	0.57
PCWP (mm Hg)	0.04	0.27	0.61
LVSWI (g·m/m^2)	0.4	4.64	0.07
%ΔCSA Ach	0.57	9.15	0.02
%ΔCBF Ach	0.68	14.9	0.006
%ΔCSA adenosine	0.20	1.72	0.23
%ΔCBF adenosine	0.20	1.74	0.23

Ach = acetylcholine; Coeff = coefficient; %ΔCBF = percent change in coronary blood flow in response to highest concentration of study drug; %ΔCSA = percent change in coronary cross-sectional area in response to highest concentration of study drug; other abbreviations as in Tables 1 and 2.

10 had fibrosis, and 5 (50%) of 10 had both. Only 1 (10%) of the 10 patients had a normal biopsy result. There was no association between biopsy findings and the coronary responses to acetylcholine or adenosine.

Clinical outcome and follow-up of left ventricular function. Follow-up clinical information was obtained for patients with DCM 16.0 ± 2.1 months (range 4 to 24) after catheterization. Two patients were free of CHF symptoms and were taking no medication; six had functional class II symptoms with standard heart failure medication; one had undergone heart transplantation; and one (Patient 7) was lost to follow-up shortly after the study. Left ventricular function was assessed 7.0 ± 1.7 months (range 1 to 16) after catheterization in nine patients with DCM. The three patients with DCM with atrial fibrillation at the time of initial study still had atrial fibrillation at the time of follow-up evaluation of left ventricular function. LVEF at follow-up had increased to 0.35 ± 0.06 (range 0.15 to 0.63; p = 0.10 vs. initial study). LVEF increased by ≥0.10 in four patients (Patients 1, 2, 9 and 10), and to >0.50 in two (Patients 1 and 2). Multiple linear regression analysis revealed that of the variables examined, only the coronary CSA and CBF responses to 10^{-6} mol/liter acetylcholine were significantly associated with improvement in LVEF (coronary CSA: r^2 = 0.57, p < 0.05; CBF: r^2 = 0.68, p < 0.01) (Table 3, Fig. 4). Patients were classified into two groups according to whether their CBF increased or decreased during infusion of 10^{-6} mol/liter acetylcholine: in group 1 (n = 4), CBF increased by 45 ± 16% (range 9% to 86%); in group 2 (n = 5) CBF decreased by 45 ± 9% (range 20% to 76%). The gender distribution was different between the groups (group 1 vs. group 2: three women, one man vs. no women, five men, p = 0.047). However, there was no difference between groups in age (56 ± 8 vs. 40 ± 7 years), duration of CHF symptoms (2 ± 1 vs. 2 ± 1 months), functional class (2.5 ± 0.3 vs. 2.0 ± 0.1), initial LVEF (0.27 ± 0.02 vs. 0.25 ± 0.04), left ventricular end-diastolic dimension (58 ± 3 vs. 72 ± 6 mm), serum sodium (138 ± 1 vs. 138 ± 2 mEq/liter); or PCWP (13 ± 4 vs. 14 ± 3 mm Hg, group 1 vs. group 2, p = NS for all). However, LVEF at follow-up was significantly greater in group 1 (0.48 ± 0.06) than in group 2 (0.24 ± 0.05, p < 0.01).

Figure 3. CSA and CBF responses to intracoronary infusion of 10^{-4} mol/liter adenosine and a 0.9% NaCl control infusion (C2) in patients with acute-onset DCM and control patients (CTRL). Adenosine infusion produced significant increases in CSA and CBF in both groups. The magnitude of the responses did not differ between DCM and control patients.



Discussion

The present study of coronary vascular responses in patients with acute-onset DCM demonstrates both an impaired coronary microvascular vasodilator response and an epicardial vasoconstrictor response to the endothelium-dependent vasodilator acetylcholine. The impaired coronary microvascular vasodilator response is demonstrated by the occurrence of a blunted CBF response in the absence of a ≥50% decrease in epicardial coronary diameter (8). Our study also demonstrates an association between preservation of coronary endothelial

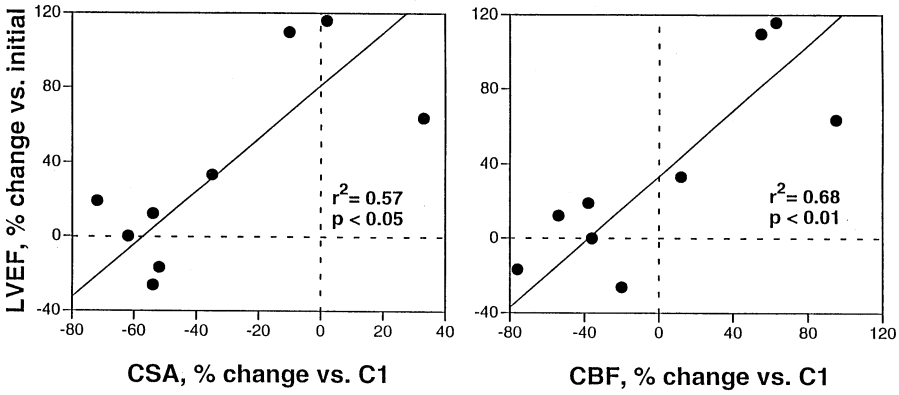


Figure 4. Plots of the relation between coronary responses to acetylcholine infusion and a 5% dextrose control infusion (C1) and subsequent improvement in LVEF in patients with acute-onset dilated cardiomyopathy. **Left,** Association between the epicardial CSA response during infusion of 10^{-6} mol/liter acetylcholine and improvement in LVEF on follow-up. **Right,** Association between the CBF response during infusion of 10^{-6} mol/liter acetylcholine and improvement in LVEF at follow-up.

function and subsequent improvement in LVEF in patients with acute-onset DCM.

Coronary endothelial dysfunction. Our finding of epicardial constriction during acetylcholine infusion in acute-onset patients with DCM has not been seen in previous studies of chronic idiopathic DCM. The magnitude of this constriction was considerable: >35% mean decrease in coronary CSA in response to 10^{-6} mol/liter acetylcholine. Paradoxical arterial vasoconstriction in response to acetylcholine administration has been previously reported in patients with hypercholesterolemia (22,23) and atherosclerosis (24), in heavy smokers (25) and in an animal model of hypertension (26). It has also been reported (27) in patients with chronic Chagas heart disease, both in those with preserved left ventricular function and those with DCM. The explanation for the finding of epicardial constriction during acetylcholine infusion is uncertain. Acetylcholine is known to cause arterial vasodilation by stimulating the synthesis and release of nitric oxide from normal endothelium. Nitric oxide subsequently diffuses to the vascular smooth muscle, where it exerts its relaxing effect through a cyclic guanosine monophosphate–mediated mechanism. Acetylcholine can also stimulate muscarinic receptor-mediated release of endothelium-derived vasoconstrictor substances, which appear to be cyclooxygenase dependent (28). Finally, acetylcholine has a direct constrictor effect on vascular smooth muscle (29). These mechanisms are in equilibrium in the normal vasculature. At lower concentrations of exogenous acetylcholine ($\leq 10^{-6}$ mol/liter), vasodilating properties predominate, whereas at higher concentrations ($>10^{-6}$ mol/liter), vasoconstriction becomes apparent (30). The epicardial vasoconstriction during acetylcholine infusion seen in our study could therefore be the result of any one or a combination of the following: the inability of abnormal endothelium to synthesize or release nitric oxide normally, enhanced direct smooth muscle constriction or augmented release of or sensitivity to a constrictor substance. A lack of smooth muscle responsiveness to endothelium-derived nitric oxide is possible, although our findings of similar increase in coronary CSA and CBF with adenosine administration in patients with DCM and control patients argue against this possibility and are in agreement with previous reports (8,10) of preserved coronary responses to endothelium-independent vasodilators in patients with DCM.

Potential role of coronary endothelial dysfunction in pathogenesis and progression of DCM. Both the etiology and the importance of coronary endothelial dysfunction in DCM remain controversial. One fundamental question is whether endothelial dysfunction is a product of the cardiomyopathy and consequent CHF or is instead a manifestation of the initial insult that produced the DCM and is therefore potentially important in the pathogenesis and progression of the myopathic state.

Several lines of evidence point to direct endothelial effects of agents that may cause DCM. Viruses known to be associated with myocarditis and DCM have been reported to infect endothelial cells (31), and autoantibodies directed against endothelium have been found in viral myocarditis (32). *Trypanosoma cruzi*, the causative agent of Chagas heart disease, induces biochemical and synthetic alterations in infected cultured endothelial cells (33,34). Finally, ethanol, a relatively common cause of DCM, has been shown to be toxic to vascular endothelium (35).

A possible role for abnormal coronary vasomotor physiology in the pathogenesis and progression of DCM is suggested by previous experimental work. Factor et al. (36) demonstrated the presence of coronary microvascular spasm in the Syrian hamster model of hereditary DCM, and a similar phenomenon was found in a murine model of acute Chagas heart disease (37). Treatment in the hamster model with a calcium channel antagonist prevented both the spasm and the subsequent development of DCM (38). To this previous evidence of direct endothelial involvement with infectious and toxic agents associated with DCM, and the potential for abnormal vascular physiology to produce myocardial dysfunction, we now add the observation of altered coronary endothelial function in patients with acute-onset DCM.

Previous studies have documented the presence of abnormal coronary endothelial function in patients with idiopathic DCM. The patients in the present study differ from those of previous studies in that the duration of symptoms in our study was specifically limited. To our knowledge there have been no previous studies of coronary physiology early in the course of human DCM. Neglia et al. (7), using positron emission tomography, found abnormalities in rest and stress myocardial blood flow in patients with mild DCM. However, the duration of

cardiac dysfunction in their study was not specified, and they may have included patients with chronic mild ventricular dysfunction rather than patients early in the course of DCM.

We also attempted to confine our study cohort to patients without an obvious cause of DCM. To this end we excluded patients with DCM and a history of significant hypertension because previous work has shown coronary (39) and systemic (40) vascular endothelial abnormalities to be present in hypertension. We also excluded patients with a history of heavy ethanol abuse because previous studies have suggested that ethanol-induced DCM may exhibit different coronary endothelial responses than other types of DCM: Treasure et al. (8) noted an unusually preserved CBF response during acetylcholine administration in one of their study patients who had a history of heavy ethanol abuse (8), and more recent experimental work indicates that although short-term ethanol exposure inhibits endothelium-dependent vasodilation (41), long-term exposure enhances it by increasing the nitric oxide synthase response to a variety of stimuli (42).

Relation of coronary endothelial function to recovery of left ventricular function. In addition to the finding of abnormal coronary endothelial function in patients with acute-onset DCM, our current study demonstrates a relation between preservation of coronary endothelial function and subsequent improvement in LVEF in these patients. Several previous studies have reported on the incidence of and predictors for recovery of LVEF in patients with DCM. The incidence of improvement has ranged from 27% to 37% (43,44). Predictors of recovery of LVEF remain controversial. Although some studies have found a shorter duration of symptoms and a less severe clinical status at presentation to predict recovery (44), others have not (43) but have found histopathologic features of endomyocardial biopsy specimens to be more useful (45). To our knowledge, the current study is the first to examine the relation between coronary endothelial function and subsequent recovery of LVEF in patients with DCM. We found that preservation of coronary endothelial function at both the epicardial (as measured by changes in CSA during acetylcholine infusion) and the microcirculatory (as measured by changes in CBF during acetylcholine infusion) level was associated with improvement in LVEF. Furthermore, LVEF improved ≥ 0.10 in all patients whose CBF increased with acetylcholine infusion but did not improve significantly in patients whose CBF failed to increase. We were unable to demonstrate an association between any of the other clinical or hemodynamic variables, including the presence or absence of fibrosis or hypertrophy on endomyocardial biopsy and improvement in LVEF. This relation between preservation of coronary endothelial function and subsequent improvement in LVEF suggests the hypothesis that an abnormal coronary endothelium plays a role in the establishment and progression of ventricular dysfunction in DCM.

Clinical implications. If the coronary endothelial abnormalities found in patients with acute-onset DCM are important in the pathogenesis and progression of DCM, they may represent a target for therapeutic intervention. The previously cited work by Factor et al. (38) suggests that in some cases, treatment with a coronary vasodilator may prevent the development of DCM. It is conceivable that the efficacy of various vasodilators in patients with DCM is due in part to their effects on the coronary circulation. Evidence for this exists for angiotensin-converting enzyme inhibitors (46), hydralazine (47) and calcium channel antagonists (48). However, given their beneficial systemic hemodynamic and, in some cases, neurohumoral actions, it will be difficult to conclusively establish a direct benefit of their coronary vasodilator effects.

Study limitations. Contrary to our hypothesis that coronary endothelial dysfunction can play an etiologic and contributory role in DCM is the possibility that the endothelial dysfunction is merely a product of the DCM, regardless of the cause. This contention is supported by a number of observations: Endothelial dysfunction has been found both in the peripheral (9,14) and the coronary (8,10,11) circulation and in patients with various types of DCM, including familial (8), postpartum (7), ischemic (9) and idiopathic DCM (8,10,11), as well as in a variety of animal models of DCM (12,13). Among these is a coronary artery ligation model of CHF in the rat (49), in which the endothelial abnormalities were not present early after coronary ligation but only after 4 weeks, when CHF was fully established.

There are several potential limitations to our present study. Despite our attempts to study a cohort early in the course of DCM, it is impossible to know whether the period of time between the onset of the disease process and the onset of symptoms was uniform in the study group. Although we may have included patients with more chronic ventricular dysfunction than their histories would suggest, it would be difficult to recruit only patients with DCM in whom the exact duration of DCM is known. Although vasoactive medications were withheld for 12 to 24 h before study, the longer acting of these agents may have residual effects on coronary endothelial function testing. Our control group, with its relatively high prevalence of atherosclerotic risk factors, including hypertension, clearly does not represent a truly "normal" cohort. We thought it appropriate to enroll as control patients only those for whom catheterization was clinically indicated. Furthermore, we would have expected the differences between groups to be even greater had the control group included truly normal subjects because the presence of these risk factors has in itself been associated with abnormal endothelial function (22,23). The methods of assessing LVEF were not completely uniform. However, most patients (seven of nine) underwent echocardiography at both initial evaluation and follow-up, and analysis of the results from the three techniques used to assess LVEF at our institution has shown good agreement. Finally, our patient cohort was small, and the association found between coronary endothelial function and improvement in LVEF will need to be confirmed in a prospective fashion in a larger series.

Conclusions. We have demonstrated the presence of abnormal coronary endothelium-dependent vasodilation early in the course of human DCM. We further showed that this abnormality exists at both the epicardial and the microcircu-

latory level. Finally, we demonstrated an association between preservation of coronary endothelial function and subsequent improvement in LVEF. The potential diagnostic and therapeutic importance of these findings in acute-onset DCM remains to be determined.

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