Atherosclerosis Impairs Flow-Mediated Dilation of Coronary Arteries in Humans

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Studies in animals have suggested that increases in blood flow result in dilation of large arteries by an endothelium-dependent mechanism. Atherosclerosis can impair endothelium-dependent vasodilation to vasoactive agents. The purpose of this study was to determine whether or not large coronary arteries in humans exhibit dilation with increases in blood flow and to test the hypothesis that this response is impaired in the presence of atherosclerosis. Graded concentrations of adenosine were infused into the distal left anterior descending (LAD) coronary artery to test the dilator response of the proximal LAD to increases in blood flow. The proximal LAD was thereby exposed to changes in blood flow, but not directly to adenosine. Ten patients with angiographically smooth proximal LAD segments (group 1) and seven patients with irregularities in the proximal LAD consistent with mild atherosclerosis (group 2) were studied. Infusions of adenosine throughout the range of 0.022 to 2.2 mg/min into the LAD produced a dose-dependent increase in estimated coronary blood flow and a mean increase of $305 \pm 27\%$ at 2.2 mg/min adenosine. At 2.2 mg/min adenosine, a striking difference ($p<0.001$) occurred between the significant flow-mediated dilation of the proximal LAD observed in group 1 ($+13.2 \pm 1.3\%$ from $2.63 \pm 0.16$ mm, $p<0.001$), and the lack of dilation in group 2 ($+1.8 \pm 1.5\%$ from $3.20 \pm 0.17$ mm, $p=NS$), despite a greater increase in coronary blood flow in group 2 ($+387 \pm 29\%$) than in group 1 ($+230 \pm 36\%$). When adjacent segments of the same arteries in these patients were directly exposed to an infusion of the endothelium-independent vasodilator nitroglycerin (16.6 g/min), angiographically smooth and irregular segments dilated similarly ($+31.1 \pm 4.3\%$ and $+33.6 \pm 4.2\%$, respectively). Thus, in humans, flow-mediated dilation was observed in angiographically normal proximal LAD segments but was markedly impaired in atherosclerotic vessels. Because the normal and mildly atherosclerotic vessels were capable of equal dilation to the endothelium-independent vasodilator nitroglycerin, the absence of flow-mediated dilation in atherosclerosis may reflect impaired endothelial vasodilator function. (Circulation 1989;80:458-465)

Dilation of large conduit arteries has been shown to occur in response to increases in blood flow. This phenomenon has been observed in isolated, perfused canine coronary artery segments in situ and during the reactive hyperemia after brief coronary artery occlusions. Holtz et al showed that flow-mediated dilation in isolated, perfused canine epicardial coronary arteries in vitro was abolished by removal of the endothelium. Recently, Inoue et al showed that flow-mediated dilation of epicardial coronary arteries in conscious dogs was also attenuated by removal of the endothelium.

The absence of intact endothelium affects the vasomotor responses to many compounds including acetylcholine, serotonin, thrombin, and ADP. Studies of isolated vessels from experimental animal models of atherosclerosis and atherosclerotic human coronary arteries have shown that endothelium-dependent relaxation is attenuated by atherosclerosis. In earlier work, we showed that although the intracoronary infusion of acetylcholine produces dilation of angiographically normal coronary arteries, it produces paradoxical vasoconstriction in patients with mild or advanced coronary atherosclerosis. The goals of the present study were to determine whether or not increases in blood flow produce dilation in normal human epicardial coronary arteries in vivo and whether or not flow-mediated dilation is impaired in the presence of coronary atherosclerosis.
Methods

Study Design

The purpose of this study was to investigate the vasomotor responses of a proximal segment of the left anterior descending (LAD) coronary artery in response to increases in coronary blood flow induced by the administration of adenosine into a middle segment of the LAD. Seventeen patients undergoing diagnostic catheterization for investigation of coronary disease were studied. Each patient was selected because the LAD showed no stenosis of 50% or greater. Patients with unstable angina, recent myocardial infarction, valvular heart disease, elevated left ventricular filling pressures, or abnormal left ventricular systolic function were excluded. Antianginal therapy was discontinued 18–24 hours before catheterization, except for the unrestricted use of sublingual nitroglycerin, which was withheld 1 hour before catheterization.

Diagnostic right and left heart catheterization and coronary angiography were performed by a standard percutaneous femoral approach. After completion of the diagnostic catheterization, an additional 5,000 units heparin were given intravenously, and an 8F guiding catheter was positioned in the ostium of the left coronary artery. A 20-MHz pulsed Doppler crystal mounted on the tip of a 2.5F infusion catheter (Millar Instruments, Houston, Texas) was advanced through the guiding catheter into the middle segment of the LAD. The use of this device to assess intracoronary blood flow velocity has been described in detail. The Doppler catheter was connected to a photographic multichannel oscillographic recorder (VR16, Electronics for Medicine, Pleasantville, New York) to display phasic and mean velocity waveforms. Before beginning the experimental protocol, the position of the Doppler flow velocity catheter and the range gate control were adjusted to optimize the audio flow velocity signal and the phasic flow velocity waveform. The Doppler catheter position and the range gate control were not changed thereafter. The Doppler catheter position was carefully checked throughout each protocol.

Protocol

Serial 2-minute intracoronary infusions of vasoactive agents dissolved in 0.9% NaCl were administered at 0.8 ml/min through the central lumen of the Doppler catheter in the following sequence: control (0.9% NaCl); graded concentrations of adenosine (Sigma Chemical, St. Louis, Missouri) (three doses throughout the range of 0.022 to 2.2 mg/min); repeat control (0.9% NaCl), and nitroglycerin (16 μg/min). Assuming a resting blood flow in the LAD of 80 ml/min, these doses of adenosine would give a final blood concentration of approximately $10^{-6}$ to $10^{-4}$ M, but with the increase in blood flow at higher doses, the actual concentrations were proportionately lower. Just before the end of each infusion, coronary arteriography was performed in biplane orthogonal views with the use of a power injection of nonionic contrast medium (Omnipaque, Winthrop-Breon, New York). Throughout each infusion, the heart rate, arterial pressure, coronary flow velocity, and electrocardiogram (lead I) were monitored continuously, and all measurements were recorded in steady-state conditions.

Classification of Patients

After catheterization, the patients were divided into two groups based on the extent of atherosclerosis on the diagnostic coronary angiograms. Classifications were made by consensus of three investigators unaware of the study results. The proximal LAD was classified as smooth or irregular.

Group 1: Controls. Ten patients had angiographically smooth proximal LAD segments. All of these patients had atypical chest pain with negative or equivocal exercise tolerance tests. There were six men and four women ranging in age from 17 to 72 years (mean, 47 years). Five of these patients had angiographically normal, smooth coronary arteries throughout, without luminal irregularities (group 1A). The other five of these 10 patients had smooth proximal segments but also had luminal irregularities in the distal LAD or in another coronary artery (group 1B).

Group 2: Mild atherosclerosis. Seven patients had luminal irregularities in the proximal LAD on diagnostic angiography and were considered to have early atherosclerosis in that arterial segment. Patients with a focal LAD stenosis of 50% or greater diameter narrowing were not studied to avoid the confusing effect of a flow-limiting stenosis. Three group 2 patients had stenoses of greater than 50% diameter narrowing in the right or circumflex coronary artery. There were six men and one woman ranging in age from 38 to 66 years (mean, 52 years).

Before the diagnostic catheterization, written, informed consent was obtained from all patients in accordance with guidelines established by the Committee for the Protection of Human Subjects at Brigham and Women’s Hospital.

Quantitative Coronary Angiography

Quantitative coronary angiography was performed by a previously validated technique. Nonionic contrast medium was injected into the left coronary artery at the rate of 5–10 ml/sec to a total of 7–12 ml with the use of a power injector (Medrad, Pittsburgh, Pennsylvania) to optimize the quality and reproducibility of the injections. A biplane system, (Polydiagnost-C, Philips Medical Systems, Shelton, Connecticut) and two image intensifiers were used. The LAD was positioned in the center of each field of view and at a single position in space (isocenter).

Analysis of Arterial Dimensions

To examine the serial reactions of the available coronary segments, the technically more suitable single-plane coronary angiograms were selected from
the biplane views for analysis. We used changes in diameter to determine vasomotor responses and used the second view (biplane view) to ensure that the same directional change occurred in more than one dimension. In 17 patients, a segment of the LAD free of side branches and at least 5 mm proximal to the Doppler catheter tip (site of infusion of vasoactive agents) was selected for quantitative analysis (Figure 1). These segments were exposed to changes in blood flow but not directly to vasoactive agents. A segment of the LAD at least 10 mm distal to the catheter tip was selected for analysis of the direct effects of the vasoactive agents. Not more than one proximal segment and one distal segment were analyzed in each patient.

In three patients from group 1 and three patients from group 2, a segment of the circumflex coronary artery not exposed to vasoactive agents and not exposed to increased blood flow was analyzed to exclude a second-pass effect of adenosine on the proximal coronary arteries.

The epicardial coronary artery segment under study was centered, and each cineframe was digitized with the use of a videocamera (Cohu, San Diego, California) connected to a video interface (Recognition Concepts, Incline Village, Nevada) and a Microvax II computer (Digital Equipment, Maynard, Massachusetts). Four cineframes were scanned and averaged with two anatomic features as references in each frame to ensure accurate repositioning. Sixteen video scans were summed to reduce video noise, and two-line profile averaging was used to minimize anatomic noise. Calibrated grids, filmed at isocenter, were used to scale the data from pixels to millimeters. A series of measurements of diameter was recorded for each pixel line for the length of each arterial segment. The data were displayed in the form of a graph showing diameter compared with length. Fixed anatomic coordinates were used to reproduce these regions of interest in repeated measurements to assess serial changes.

**Estimates of Coronary Blood Flow Changes**

Estimates of changes in coronary blood flow were made by correcting changes in mean coronary blood flow velocity, as measured directly by the Doppler catheter, for changes from control in estimated vessel cross-sectional area at the catheter tip as determined from the change in diameter measured in the optimal single-plane view.¹⁷

**Statistical Analyses**

The four digitized cineframes for each infusion were summed and averaged along the segment profile to give a mean diameter and standard deviation at each point. A single mean and a pooled standard deviation for the segment (at each infusion) was obtained by averaging each of these measures along the segment profile. Suitable segments were required to have a mean standard deviation of less than 5% of the mean diameter. Results for multiple adenosine doses were evaluated by analysis of variance, and Bonferroni's *t* tests were applied to determine which values were different from baseline. Group differences were analyzed by unpaired *t* tests. Within the range of changes in coronary blood flow examined in this study, a linear relation was found between coronary artery diameter and coronary blood flow in each patient. The slope of the regression was calculated for each patient, and the group differences of the slopes were compared with Wilcoxon's ranked-sum tests for unpaired data.

Statistical significance was assumed if the null hypothesis (two-tailed) could be rejected at the 0.05 probability level. All data are expressed as the mean±SEM.

**Results**

**Coronary Flow Responses**

Infusion of adenosine throughout the range of 0.022 to 2.2 mg/min into the LAD produced a dose-dependent increase in estimated coronary blood flow. At 0.022 mg/min, group 1 (smooth segments)
showed a 96±25% increase in coronary blood flow, and group 2 (irregular segments) showed a 223±79% increase in coronary blood flow. At 0.22 mg/min, groups 1 and 2 showed 198±47% and 341±59% increases in coronary blood flow, respectively. At the peak dose of adenosine (2.2 mg/min), the increase in estimated coronary blood flow was somewhat greater in patients with angiographically irregular proximal segments (group 2) (+387±29%) than in patients with angiographically smooth proximal segments (group 1) (+230±36%).

**Systemic Effects**

At the peak dose of adenosine (2.2 mg/min), heart rate did not change significantly from baseline in group 1 (+4.4±2.1 from 64±2.8 beats/min) or in group 2 patients (−1.1±1.7 from 68±4.1 beats/min). Mean arterial pressure increased equally by 17.5±2.8 from 95±4.7 mm Hg in group 1 patients and by 11.3±2.1 from 103±4.7 mm Hg in group 2 patients (p=NS). No significant baseline differences were found in heart rate or blood pressure between group 1 and 2 patients. Two patients experienced flushing, two developed mild dyspnea, and one experienced mild chest fullness. These symptoms abated promptly after discontinuation of the adenosine infusion.

**Flow-Mediated Dilation of Epicardial Coronary Arteries**

The relation between change in coronary artery diameter and change in coronary blood flow for group 1 and 2 patients is shown in Figure 2. The average slope of the regression lines describing this relation in group 1 patients was 0.064±0.008 (6.4% dilation for each fold increase in flow) but was strikingly reduced (p<0.01) to 0.005±0.003 (0.5% dilation for each fold increase in flow) in group 2 patients. At the maximum dose of adenosine (2.2 mg/min), a striking difference (p<0.001) occurred between the flow-mediated dilation observed in group 1 and the lack of dilation in group 2 patients: the proximal LAD dilated by 13.2±1.3% from 2.63±0.16 mm in group 1 patients (p<0.001) but did not change in group 2 patients (+1.8±1.5% from 3.20±0.17 mm, p=NS) (Figure 3).

There was a small difference in the baseline coronary diameters between the two groups, with a baseline diameter of 2.63±0.16 mm in group 1 patients and 3.20±0.17 mm in group 2 patients. By linear regression, there was no correlation between baseline diameter and dilation in group 1 patients at 2.2 mg/min adenosine (r = -0.10) or between baseline diameter and the slopes of the regression lines describing the relation of coronary blood flow and diameter for each patient in group 1 (r = -0.18). Although the number of subjects in each group is too small to perform meaningful subset analysis, examination of only those vessel segments with a baseline diameter of 2.5 mm or greater revealed essentially the same results, with a 12.8±2.0% increase in the five patients in group 1 and a 0.6±1.0% increase in the six patients in group 2 meeting this criterion. In those vessels over 3.0 mm in diameter at baseline, the increase was 12.9% in the two patients in group 1 and 0.2% in the five patients in group 2 meeting this criterion. Thus, there is no evidence to suggest that this small difference in baseline diameter between groups 1 and 2 accounts for the marked difference in dilation with the increased flow that was observed. No difference was found between the dilation observed in group 1A (+13.6±2.4%) and group 1B (+12.7±1.3%).

There was no dilation of the left circumflex coronary artery segments in six of the patients during LAD adenosine infusion, thus excluding a significant direct second-pass effect of adenosine. In fact, a slight reduction in diameter of the left circumflex coronary artery segments was observed with LAD adenosine infusions (−5.1±0.6%), and no difference occurred between group 1 (−5.0%) and group 2 patients (−5.3%).

**Direct Drug-Mediated Dilation of Epicardial Coronary Arteries**

In response to the endothelium-independent vasodilator nitroglycerin, angiographically normal seg-
ments (group 1) distal to the Doppler catheter tip, but adjacent to the proximal segment, dilated +31.1 ± 4.3% from 1.96 ± 0.17 mm, p < 0.001. Atherosclerotic segments (group 2) distal to the Doppler catheter tip, but adjacent to the proximal segment, dilated by an equal amount in response to nitroglycerin, +33.6 ± 4.2% from 1.73 ± 0.13 mm, p < 0.001.

We also observed that the nitroglycerin infusion of 32 μg for 2 minutes into the mid LAD caused dilation of the circumflex coronary artery and a slight decrease in systolic blood pressure (4.5 ± 1.9 mm Hg), suggesting recirculation of a fraction of the nitroglycerin. As a consequence, the smooth and irregular proximal LAD segments dilated equally during nitroglycerin infusion (10.3 ± 2.5% in group 1 and 8.0 ± 2.4% in group 2). These results showed that both the smooth and atherosclerotic proximal segments were capable of dilation and that both dilate equally to a low dose of recirculating nitroglycerin.

In response to adenosine (2.2 mg/min) smooth coronary segments distal to the Doppler catheter tip in group 1 patients dilated +28.3 ± 4.9% from 1.96 ± 0.17 mm, p < 0.01, and segments with mild atherosclerosis in group 2 patients dilated to a similar degree, +29.8 ± 3.9% from 1.73 ± 0.13 mm, p < 0.01.

**Discussion**

This study in conscious patients shows that angiographically normal epicardial coronary arteries in humans respond to increases in blood flow with vasodilation and that this reaction is impaired in the presence of mild atherosclerosis.

In 1933, Schretzenmayr described dilation of the in situ canine femoral artery in response to increases in blood flow. Hilton proposed that the mechanism of this dilation involved "propagated vasodilation" from the arteriolar to the larger proximal arteries. However, later experiments failed to show inhibition of this response by transection of the femoral artery distal to the site of measurement of femoral artery dimensions, thus implicating a local control mechanism rather than a propagated wave of vasodilation. The sensor mechanism for flow-mediated dilation was unclear until Holtz et al. showed that flow-mediated dilation in isolated, perfused canine epicardial coronary arteries in vitro was abolished by removal of the endothelium. Recently, Inoue et al. showed that flow-mediated dilation of epicardial coronary arteries in intact, conscious dogs was also attenuated by removal of the endothelium.

Furchgott and Zawadzki first showed that dilation of arteries in response to acetylcholine is dependent on the presence of an intact endothelium. Subsequently, removal of the endothelium was shown to alter the vasomotor responses to many other compounds, including serotonin, ADP, and thrombin. A number of studies have shown that endothelium-dependent relaxation is mediated by the release from endothelial cells of a diffusible, chemically unstable substance(s), referred to as...
endothelium-derived relaxing factor (EDRF), the precise identity of which is still under investigation. Rubanyi et al found that increases in arterial flow will trigger the release of a vasodilating substance from endothelial cells that has characteristics similar to the EDRF released by acetylcholine.

Recent studies showed that atherosclerosis can have a profound effect on endothelium-dependent vasomotor responses, whereas the response to nitroglycerin remains intact. In a primate model of atherosclerosis, Freiman et al found depressed relaxation in iliac arteries in response to acetylcholine and thrombin compared with arteries from normal animals, whereas the responses to the endothelium-independent dilator nitroglycerin were similar. Bossaller et al and others have studied the responses of normal and atherosclerotic coronary arteries in the explanted hearts from cardiac transplant recipients and found that relaxation to acetylcholine was abolished in atherosclerotic arteries. A previous study from our catheterization laboratory showed that intracoronary infusion of acetylcholine produced dilation of angiographically smooth coronary arteries. However, paradoxical constriction was observed in atherosclerotic arteries, even in those with mild disease.

The present study extends these findings in humans by showing that flow-mediated dilation of large coronary arteries occurs in humans and is impaired in atherosclerosis. That this abnormality represents endothelial dysfunction rather than a more general impairment of epicardial artery dilator capacity is suggested by the observation that these atherosclerotic vessels responded normally to nitroglycerin, an agent that directly relaxes vascular smooth muscle. These findings are consistent with the existence of a more generalized dysfunction of the endothelium in human atherosclerosis rather than an isolated muscarinic defect.

A “smooth” appearance of the luminal surface on the coronary angiogram does not exclude the presence of intimal involvement with atherosclerosis. Therefore, a limitation of this study concerns the ability to distinguish atherosclerotic from “normal” coronary arteries. However, in this study, the reactions to increases in coronary blood flow were strikingly different when reviewers unaware of the study results separated patients with smooth coronary arteries from patients with luminal irregularities.

We chose adenosine as a vasodilator in this study primarily because it elicits dose-dependent and near maximal dilation of coronary resistance vessels and because it has a half-life of less than 10 seconds in the circulation, making it ideal for quickly achieving steady-state levels and a local effect with a constant infusion. Preferably, measurements of flow-mediated dilation should be performed under conditions as close to steady state as possible because of the dissociation of the time of peak flow and the time of maximal large vessel flow-mediated dilation after brief increases in flow.

In the present study, we measured coronary blood flow velocity by means of an intracoronary Doppler catheter. This particular system has been validated in animal experiments. The advantages of this system are that it provides instantaneous and continuous measurement of phasic flow velocity and allows measurement well beyond the left main coronary artery without significantly restricting maximal blood flow. The estimation of volume blood flow in this study is affected by coronary artery cross-sectional area, and we therefore adjusted the flow velocities for changes in cross-sectional area at the site of the Doppler sample volume to provide an index of volume flow. This study was not concerned with single absolute measures of volume blood flow but was concerned with serial changes using each patient as his own control. Of note, the flow-mediated dilation seen in group 1 patients was not due to greater increases in flow. Indeed, group 2 patients developed even greater increases in flow with adenosine.

A pressor response with the intravenous administration of large doses of adenosine has been described in conscious humans. In the present study, at the highest dose of intracoronary adenosine, blood pressure increased significantly and equally in groups 1 and 2. The mechanism of this response is under investigation at present. Although this increase in distending pressure may have augmented the apparent flow-mediated dilation in group 1 patients to a small degree, it should not account for the marked differences between group 1 and 2 patients because the increases in blood pressure in those groups were similar.

One potential limitation of this study is the possibility that some of the adenosine infused through the Doppler catheter had diffused retrograde to the proximal arteries. Chilian and Marcus have shown in open-chest, anesthetized dogs that retrograde blood flow does occur in mid-to-late systole in intramural and small epicardial arteries just before they penetrate into the myocardium. Retrograde flow was not observed in the LAD artery during any phase of the cardiac cycle. In addition, during marked increases in coronary blood flow produced by intravenous dipyridamole, only antegrade flow was observed in the LAD. Because we infused adenosine into the LAD artery at a rate of approximately 1% (0.8 ml/min) of the estimated LAD blood flow (80 ml/min), there is no reason to believe that retrograde diffusion occurred. Also of note, there were no negative systolic coronary flow velocities in the phasic Doppler signal recordings at baseline or during adenosine infusion. Finally, in the atherosclerotic arteries, there was no dilation of the proximal segment and marked dilation of the adjacent segment distal to the Doppler during adenosine infusion. This finding also strongly argues against retrograde diffusion in this experimental design.
Another potential limitation of the study concerns the possibility that a supramaximal dose of intracoronary nitroglycerin could mask a real difference between the two groups in their responsiveness to this endothelium-independent vasodilator. However, Feldman et al\textsuperscript{29} have described a dose-response curve for intracoronary nitroglycerin, and our 32-\textmu g dose was near maximal but clearly not supramaximal. At a dose of 400 \textmu g sublingually, Feldman et al\textsuperscript{30} found no difference between the dilations produced in patients with "normal" arteries and those produced in patients with coronary artery disease when obviously stenotic segments were excluded. In addition, studies of isolated vessels from normal and atherosclerotic monkeys have shown no difference in dose-response curves for relaxation and response to nitroglycerin.\textsuperscript{9}

Clinical Implications

With basal levels of blood flow, large conduit arteries contribute only a small fraction to the total resistance in a given vascular bed. At higher flow rates, the pressure drop in traversing large vessels can increase, and this is minimized by flow-mediated dilation. Dilation of a vessel with an increase in flow also tends to minimize flow velocity and shear stress for a given volume flow, perhaps serving to protect endothelial cells from damage that may occur with exposure to high shear stress. In vessels with atherosclerotic stenoses, the large conduit vessels contribute more to total vascular resistance as narrowing increases. In the presence of a stenosis, increases in flow can be limited. If such narrowed segments were capable of dilating in response to increases in flow, substantial reductions in resistance could occur, but the impairment of flow-mediated dilation in atherosclerotic vessels eliminates this potentially beneficial mechanism. Stimuli encountered in daily life such as exercise and the sympathetic stimulation produced by the exposure to cold normally increase coronary blood flow.\textsuperscript{17,31} Previous studies showed that angiographically normal coronary arteries dilate in response to exercise and the cold pressor stimulus, but even mildly atherosclerotic vessels exhibit constriction under the same conditions.\textsuperscript{17,32,33} Although the lack of a dilator response of these atherosclerotic vessels is probably multifactorial, the data in this study indicate that the loss of flow-mediated dilation could represent a major component.

In summary, this study shows that in conscious humans angiographically smooth epicardial coronary arteries exhibit dose-dependent dilation in response to increasing blood flow. This response is grossly impaired in the irregular coronary segments of patients with angiographic evidence of atherosclerosis. Because dilation in response to the endothelium-independent vasodilator (nitroglycerin) remains intact, this failure of dilation is likely due to endothelial dysfunction.

References


KEY WORDS • adenosine • endothelium • atherosclerosis • arteries • vasodilation
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