Early Evidence of Endothelial Vasodilator Dysfunction at Coronary Branch Points

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Intracoronary acetylcholine produces endothelium-dependent dilation of normal coronary arteries and paradoxical constriction of atherosclerotic vessels. Regional differences in endothelium-dependent vasomotion, however, have not been studied in relation to the nonuniform development of atherosclerosis. We compared the vasomotor response to increasing doses of acetylcholine of angiographically smooth coronary artery segments prone to atherosclerosis (coronary branch points) with segments remote from branch points (straight segments). In patients with entirely smooth coronary arteries and a dilator response to acetylcholine (group 1, n=7), branch points and straight segments demonstrated equal and significant dose-dependent dilation to acetylcholine (14.7±8.9% and 12.3±12.7%, respectively; p=NS). In patients with early atherosclerosis as manifest by luminal coronary irregularities, the lowest dose of acetylcholine (10-8 M) produced constriction at branch points and slight dilation at straight segments (−6.3±7.4% vs. +2.2±7.3%, p<0.05). At higher doses of acetylcholine, both branch point and straight segments constricted, but constriction remained more pronounced at branch points. Both branch point and straight segments, however, retained the ability to dilate to the non-endothelium-dependent agent, nitroglycerin. In a third group of patients with angiographically entirely smooth coronary arteries but without dilation to acetylcholine, constriction to acetylcholine again occurred first at branch points. Thus, coronary branch points demonstrate increased sensitivity to acetylcholine-induced constriction in patients with angiographic evidence of early coronary atherosclerosis and in middle-aged patients with smooth coronary arteries. These segments, however, retain the ability to dilate to nitroglycerin. Whether this early evidence of defective endothelium-dependent vasodilation predicts the later development of occlusive atherosclerosis is not yet known. (Circulation 1990;82:1169–1173)

Coronary atherosclerosis has a long asymptomatic phase of development, and only the later phases, particularly segmental narrowing and stenosis, can be assessed by coronary angiography. Furthermore, atherosclerosis is a focal disease with specific sites of predilection that include branch points and other regions of disturbed flow. Experimental studies suggest that alterations in local arterial wall shear stress produce morphological changes in the vascular endothelium that may play a central role in atherogenesis.

The vasodilator function of the endothelium can be determined by assessing the response to local acetylcholine (ACH) administration. Vascular responses to ACH are believed to be the result of two opposing actions, direct stimulation of vascular smooth muscle and release from the endothelial cells of an endogenous vasodilator, called the endothelium-dependent relaxing factor (EDRF). Abnormal endothelium-dependent vasomotion, as defined by a constriction to local ACH, has been demonstrated both in atherosclerotic vessels and in angiographically smooth segments of patients with coronary atherosclerosis. Regional differences in endothelium-dependent vasomotion, however, have not been studied in human coronary arteries with respect to the nonuniform development of atherosclerosis.

The aim of this study was to investigate by quantitative angiography segmental differences in endothelial vasodilator function by comparing the vascular...
response to ACH, and the response to the direct smooth muscle relaxant, nitroglycerin, of apparently normal arterial segments that are predisposed to development of atherosclerosis (coronary branch points) with segments remote from branch points in patients with and without coronary atherosclerosis.

**Methods**

**Patient Selection**

Patients undergoing diagnostic cardiac catheterization for investigation of coronary artery disease were studied. Patients with unstable angina, recent myocardial infarction, valvular heart disease, congestive heart failure, or severe peripheral vascular disease were not studied. Two groups of patients (entirely smooth coronary arteries and irregular coronary arteries) were defined by vessel morphology, based on the consensus of three observers who were unaware of the vasomotor responses; those patients with entirely smooth coronary arteries were then subdivided into two groups on the basis of their vasomotor response as described herein. The responses to ACH of some of these patients have been described elsewhere. This study, however, addressed an entirely different question and involved reanalysis of all angiograms de novo.

**Study Design**

Informed consent was obtained from all subjects, and the protocol was approved by the Committee for the Protection of Human Subjects at Brigham and Women's Hospital. Antiplatelet therapy was discontinued at least 16 hours before cardiac catheterization. After completion of the diagnostic catheterization, a 3F infusion catheter (Millar Instr., Inc., Houston, TX) was advanced through a standard angioplasty-guiding catheter to the proximal left anterior descending artery. Serial infusions were then delivered by a Harvard pump (Harvard Apparatus, South Natick, Mass.) at a rate of 0.8 ml/min in the following sequence: 1) 2-minute control infusion (5% dextrose in water), 2) three 2-minute infusions of ACH giving final intracoronary concentrations of approximately 10⁻⁹, 10⁻⁷, and 10⁻⁵ M, assuming a left anterior descending artery blood flow of approximately 80 ml/min, 3) 2-minute infusion of nitroglycerin (40 μg). All patients received all three infusions of ACH unless a submaximal dose produced pronounced constriction; if this occurred, subsequent doses were omitted. At the end of each infusion, coronary arteriography was performed using 8–9 ml nonionic contrast medium (Omnipaque 350, Winthrop-Breon Laboratories, N.Y.) injected at 5–7 ml/sec using a power injector (Medrad Mark IV, Medrad, Pittsburgh).

**Segment Selection**

In each patient, four coronary segments were selected for analysis from the control angiogram, two adjacent to major branch points and two remote from any visible branch points on straight segments of the left anterior descending artery. For the purposes of edge detection, the “branch point segment” began just distal to the flow divider (i.e., the point at which flow separation first occurs at the branch point) and extended distally for 2–3 mm. The straight segment of each pair was of equivalent length. Averaged values for the two branch point and two straight segments in each patient were then calculated and used for the subsequent statistical analysis.

**Quantitative Angiography**

Technically suitable single-plane angiograms were selected for analysis from the biplane views by consensus of three investigators. The coronary artery segment of interest was digitized at 20–40 μm per pixel by using a video camera (Cohu, San Diego, Calif.), a video interface (TRAPIX SS 92U, Recognition Concepts, Inc., Reno, Nev.), and a Microvax II computer (Digital Equipment, Maynard, Mass.). Four cine frames were scanned and averaged, two fixed anatomical features serving as references to ensure accurate alignment. Sixteen video images of each cine frame were summed to reduce video noise, and two-line profile averaging was used to minimize anatomical noise.

The two fixed anatomical features were also used to ensure accurate registration between different infusions and, therefore, to allow assessment of serial and changes in the same arterial segment. By using an edge-detection algorithm, a series of measurements of diameter along the length of the arterial segment was derived for each pixel line and mean diameter of the arterial segment calculated for each infusion. If, however, the standard deviations of diameter at each pixel line exceeded 10% of the mean value, suggesting pronounced variability in the interpretation of consecutive cine frames, those frames were reanalyzed. If this variability persisted, they were excluded.

**Study Groups**

Twenty-five patients were studied. Initially, two groups were derived on the basis of vessel morphology, that is, patients with entirely smooth coronary arteries (n=15) and patients with luminal irregularities (n=10), based on the consensus of three observers who were unaware of the vasomotor responses. Those patients with entirely smooth coronary arteries were subdivided according to their vasomotor response. Thus, if the four segments tested demonstrated dilation in response to ACH, the patient was considered a smooth dilator; if the responses included constriction of one or more segments by at least 5%, the patient was considered a smooth constrictor. Thus, three groups were derived.

**Group 1.** Group 1 comprised patients with smooth coronary arteries and a dilator response to ACH ("smooth dilators"). Seven patients (three males and four females) with entirely smooth coronary arteries exhibited dilation to ACH. Mean age was 37.4 years (range, 17–58 years).
Group 2. Group 2 comprised patients with smooth coronary arteries and a constrictor response to ACH ("smooth constrictors"). Eight patients (four men and four women) with entirely smooth coronary arteries demonstrated constriction of at least 5% in at least one segment in response to ACH. Mean age was 49.5 years (range, 30–72 years).

Group 3. Group 3 comprised patients with luminal irregularities ("atherosclerotic"). Ten male patients formed the atherosclerotic group. All had luminal irregularities of the left anterior descending artery but not of the test segments and no focal stenosis of 30% or greater. Seven also had a significant stenosis (>70%) of the circumflex or right coronary artery. Mean age for this group of patients was 55.4 years (range, 47–68 years).

Statistical Analysis
All data are expressed as mean±SD. Changes from control diameter were calculated with a paired t test, and comparisons between branch point and straight segments were made with a paired t test by using the Bonferroni correction for multiple comparisons. Because comparisons were made at three different concentrations of ACH, statistical significance was assumed if the null hypothesis could be rejected at the 0.02 probability level.

Results
For patients in group 1 (smooth dilators), the baseline diameter of the branch points was 1.8±0.1 mm, and the baseline diameter of the straight segments was 1.9±0.1 mm (p=NS). Figure 1 summarizes the response of the branch point and straight segments in this group and demonstrates that branch point segments and straight segments show equal and significant dose-dependent dilation to ACH. Additionally, branch point and straight segments dilated equally to the non–endothelium-dependent agent, nitroglycerin (+34.1±14.1% vs. +31.0±16.2%, p=NS).

In the patients with atherosclerosis (group 3), the mean baseline diameter of the branch point segments was 1.9±0.1 mm and of the straight segments was 2.0±0.2 mm (p=NS). In this group, however, branch point and straight segments behaved differently (Figure 2). At 10⁻⁸ M ACH, straight segments dilated slightly, whereas branch point segments constricted (+2.2±7.3% vs. −6.3±7.4%, p<0.05). At 10⁻⁷ M ACH, constriction was seen at both straight and branch point segments, although the degree of constriction remained greater at branch points (−25.2±10.8% vs. −15.5±14.2%, p<0.05). At the maximal dose of ACH (10⁻⁶ M), there was still a tendency for greater constriction at branch points, although this difference was no longer statistically significant (−25.3±19.9% vs. −17.1±18.7%, p=NS). In response to nitroglycerin, however, both branch point and straight segments dilated and dilated equally (+11.7±13.5% vs. +16.9±21.8%, p=NS).

For the patients with angiographically smooth coronary arteries but with at least one segment showing constriction to ACH (group 2, smooth constrictors), there was again no difference in mean baseline diameter between branch and straight segments (2.2±0.2 mm and 2.2±0.2 mm, respectively; p=NS). Although constriction was less pronounced than in the group of patients with atherosclerosis, the directional response was similar; thus, branch points again showed more constriction than straight segments to ACH, although this was significant only at 10⁻⁷ M ACH (Figure 3). In this group, nitroglycerin again produced equal and significant dilation at branch point and straight segments (+21.3±17.7% vs. +17.6±12.5%, p=NS).
Discussion

In this study, angiographically smooth coronary branch points from patients with atherosclerosis appear to be more sensitive to ACH-induced constriction than segments remote from branch points. This is, however, not attributable to structural disease preventing the branch point segment from dilating because the normal dilator response to nitroglycerin is preserved. Nor does it appear that the local anatomy of the branch point prevents dilation because branch point and straight segments from patients with entirely smooth coronary arteries and a dilator response to ACH were able to dilate equally. Furthermore, angiographically smooth branch points from patients with no evidence of atherosclerosis but with a constrictor response to ACH also show increased sensitivity to ACH-mediated constriction.

Furchgott and Zawadski\textsuperscript{17,2} demonstrated that arterial strips exhibit endothelium-dependent dilation in response to a specific dose range of ACH. In vivo studies during cardiac catheterization have shown that a similar estimated dose range of ACH elicits dilation of angiographically normal coronary arteries in the majority of patients with no evidence of atherosclerosis.\textsuperscript{12,16} The same studies have shown that ACH almost invariably elicits constriction in patients with angiographic evidence of early or late atherosclerosis.\textsuperscript{11,12,16} Our results are at least consistent with the hypothesis that within a particular dose range, dilation to ACH is the normal response of healthy arteries, and that constriction is indicative of endothelial dysfunction and perhaps early atherosclerosis.

The proximal coronary arteries and particularly their branch points are sites of predilection for the development of atherosclerosis.\textsuperscript{3} Thus, in the presence of established risk factors, local anatomy plays a role in the formation of lesions. That such local factors might act primarily on the endothelium was suggested by studies showing that arterial branch points demonstrated increased endothelial cell turnover\textsuperscript{4} and focal endothelial damage including small areas of complete endothelial denudation, by both light microscopy\textsuperscript{5} and scanning electron microscopy.\textsuperscript{6}

We can speculate that one such "local risk factor" might be shear stress. Shear stress is the frictional force acting per unit area on the vessel wall and is dependent on both the viscosity of the blood and the velocity gradient at the wall.\textsuperscript{17} The finding that endothelial cells are sensitive to shear stress\textsuperscript{18} and are damaged by exposure to high shear stress\textsuperscript{19} led to the suggestion that atherosclerosis might develop at sites where high shear stress damaged endothelial cells.\textsuperscript{20} Careful pathological studies, however, have shown that the distribution of atheromatous lesions does not correspond to areas of high shear stress.\textsuperscript{21} For example, at the bifurcation of the human carotid artery, it has been shown that intimal thickness is greatest along the lateral wall where shear stress is low and is less developed along the medial (flow divider) wall where shear stress is high\textsuperscript{22}; indeed, two further studies have demonstrated a powerful negative correlation between maximal shear stress and intimal thickness.\textsuperscript{23,24} Nevertheless, flow characteristics other than estimated maximal wall shear may be important. Flow at the lateral wall of a branch point can be characterized by the loss of unidirectional laminar flow with the development of turbulence. Interestingly, the turnover rate of cultured endothelial cells is increased, suggesting possible cell injury, by exposure to levels of turbulent shear stress that are much lower than those required for laminar shear stress.\textsuperscript{25} Thus, although high shear stress can damage endothelial cells, in vivo a loss of normal laminar flow, flow separation, and turbulence with rapidly changing shear stress may be the more common damaging force.

From our data, we cannot localize an abnormality of endothelium-dependent vasodilation to the flow divider or to the lateral wall of a bifurcation. Additionally, there are no data relating to the effects of other vasoconstrictors at branch points. Our findings, however, suggest that arterial segments that are predisposed to the development of atherosclerosis show early evidence of defective endothelium-dependent vasodilation in both patients with and some patients without angiographic evidence of atherosclerosis.

Whether early loss of normal endothelial function predisposes to or is simply a manifestation of early atherosclerosis is not known. Further studies are required to determine whether early evidence of endothelial dysfunction predicts the later development of occlusive atherosclerosis.

References


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