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Clinical Investigations

Impaired Endothelial Function in Patients with Rapidly Stabilized Unstable Angina: Assessment by Noninvasive Brachial Artery Ultrasonography

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Summary

Background: Endothelial dysfunction may contribute to symptoms of instability in patients with acute coronary syndromes. High-resolution external ultrasound assessment of the brachial artery responses allows noninvasive determination of endothelial function.

Hypothesis: This study was conducted to assess endothelial function in patients with unstable angina using a noninvasive technique.

Methods: We studied 189 patients who were subdivided into three groups. Group 1: 60 apparently healthy subjects with no cardiovascular risk factors or symptoms of coronary artery disease; Group 2: 105 subjects with cardiovascular risk factors—arterial hypertension, hypercholesterolemia, cigarette smoking, diabetes, and obesity, but no evidence of coronary artery disease; and Group 3: 24 patients with unstable angina (chest pain at rest within the 24 h preceding study entry). All patients underwent pre- and postischemic brachial artery test evaluation with measurements of internal arterial diameters and blood flow.

Results: Results are expressed as percentage change from basal values. Subjects in Groups 1 and 2 showed a diameter increase of 19.1 and 11.9%, respectively, whereas patients in Group 3 showed a diameter change of 1.2% ($p < 0.002$ and < 0.0001, respectively). Calculated blood flow did not differ significantly in Groups 1 or 2 (74.4 and 56.4%), but was notably lower in Group 3 (18.4%, $p < 0.005$ vs. Groups 1 and 2). In nine patients of Group 3, the brachial studies were repeated 4 weeks after symptom stabilization and showed values comparable with those in Group 2.

Conclusions: Patients with unstable angina showed endothelial dysfunction compared with control individuals. It is of interest that in patients whose symptoms were stabilized by medical therapy, endothelial function was restored 4 weeks after hospital discharge.

Key words: atherosclerosis, endothelial dysfunction, coronary disease, unstable angina

Introduction

For many decades, risk assessment and clinical management of coronary artery disease have mainly focused on the anatomical features of coronary artery disease, that is, angiographic stenosis, severity, and number of coronary vessels involved. In recent years, however, it has become apparent that dynamic aspects of vascular physiology, mainly the role of the endothelium, play a major role in both the pathogenesis and clinical manifestations of coronary artery disease.[1] Endothelial dysfunction, which results in reduced availability of nitric oxide (NO), contributes not only to atherogenesis but also to rapid coronary disease progression and acute coronary events.[2] Experimental and clinical studies have shown that NO plays an important antiatherogenic role, and in its absence, the genesis and progression of atherosclerosis are facilitated.[3] The loss of NO protective actions leads to increased release of vasoconstrictor substances, monocyte recruitment into the arterial intima, expression of surface adhesion molecules, and the production of growth factors that promote vascular smooth muscle cell proliferation and migration. Enhanced thrombogenicity and decreased fibrinolysis also result from endothelial dysfunction.[2–4]

In patients with unstable angina, endothelial dysfunction may contribute to the pathogenesis of the condition and the patients' clinical instability. Assessment of coronary endothelial function in patients with unstable syndromes creates difficult logistic problems.[5,6] Recently, noninvasive techniques using high-resolution external ultrasound have been developed that allow the in vivo assessment of brachial artery endothelial-dependent responses.[7] Findings in the brachial artery using this

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technique have been shown to correlate with coronary responses as assessed by angiography.[8,9]

The aim of the present study was to compare endothelial-dependent and -independent vasodilator responses of the brachial artery in patients with unstable angina, apparently healthy subjects with one or more coronary risk factors, and normal controls.

Methods

Patients and Controls

In all, 24 patients with unstable angina and 165 apparently healthy control individuals were divided into three groups. Group 1 (control group) consisted of 60 apparently healthy subjects, 32 men and 28 women, aged 44.8 ± 17.4 years (range 17–80) without cardiovascular risk factors. All underwent clinical, electrocardiographic, exercise stress test, echocardiographic, and laboratory test examination, which showed normal responses. These patients were normal volunteers recruited among members of the hospital staff. None of these subjects was receiving cardiovascular medication. Group 2 (risk factor group) consisted of 105 asymptomatic subjects, 55 men and 50 women, mean age 56.4 ± 14.6 years (range 25–85). All of these subjects had at least one risk factor for coronary artery disease (hypercholesterolemia, cigarette smoking, arterial hypertension, obesity, and diabetes), but had no abnormal findings on clinical, electrocardiographic, exercise stress test, echocardiographic, and laboratory test assessment. None of these patients was receiving cardiovascular medications, lipid-lowering drugs, or antihypertensive drugs. Group 3 (unstable angina group) consisted of 24 patients, 22 men and 2 women, with a mean age of 57.5 ± 5.6 years (range 47–72). All patients with the exception of two had risk factors for coronary artery disease and all had chest pain and electrocardiographic changes at rest suggestive of myocardial ischemia within the 24 h preceding study entry. In all cases, the chest pain and electrocardiographic shifts were relieved by nitroglycerin (intravenous or sublingual) within 20 min, and patients remained asymptomatic thereafter. None of the patients showed increased serum cardiac enzymes.

The study was approved by the Ethics Committee, Teaching and Research Department, of the Hospital Militar Central, Buenos Aires, Argentina, and all patients gave written informed consent.

Risk Factors

Patients were considered to have cardiovascular risk factors if their low-density lipoprotein (LDL) cholesterol plasma level was ≥ 4.12 mmol/l (160 mg/dl) and/or fasting triglycerides > 0.8 mmol/l [none of the patients had triglycerides > 3.97 mmol/l (350 mg/dl)], if they smoked (≥ 1 cigarettes/day), had systemic arterial hypertension (defined as arm-cuff arterial blood pressure ≥ 140/90 mmHg), were obese [body mass index > 30 (kg/m^2)]; or had diabetes mellitus (fasting blood glucose > 140 mg/dl). Of the 105 subjects in Group 2, 48 had only one risk factor, 30 had two, 22 had three, one had four, and 4 had five risk factors. Patients with myocardial infarction, unstable angina, stroke, transient ischemic attacks, uncontrolled hypertension, coronary artery bypass graft, or percutaneous transluminal coronary angioplasty within the previous 6 months were excluded.

Protocol

All patients and controls underwent postischemic brachial artery test evaluation. The procedure used in the study was similar to that described previously.[7,10] Upon arrival in the coronary care unit, subjects were asked to rest in the supine position for at least 10 min. The brachial artery internal diameter was assessed at end-diastole (timed by the QRS complex) and arterial flow was measured using the pulse Doppler sample volume at ≤ 70° angle in the center of the artery [7.5 MHz transducer, attached to a standard Toshiba 140 display (Japan)]. Forearm ischemia was caused by inflating a pneumatic arm cuff up to 200 mmHg (or 30 mmHg higher than the systolic arterial pressure of the subject) for 5 min. The cuff was then deflated, the arterial flow was immediately recorded, and the arterial diameter was measured at 30 and 60 s after deflation. Ten to 15 min later, 0.3 mg of sublingual nitroglycerin were administered and measurements were repeated 2 min later. Caution was taken to image the same portion of the brachial artery throughout the study. Three cardiac cycles were analyzed and measurements were averaged. Flow was calculated from Doppler velocity and vessel diameter, by the formulae: mean flow velocity × arterial area ($\pi \times \text{radius}^2$). Results were expressed as a percentage of change of the baseline values. Subjects did not receive vasoactive drugs and had not smoked or drunk coffee or other caffeine-rich beverages for 12 h preceding testing. All patients had been completely stable and free from chest discomfort or pain for at least 12 h. Nine patients from Group 3 underwent repeat brachial testing 4 weeks after discharge while they were on no vasoactive medications for 24 h. Of these nine patients, one had no cardiovascular risk factors, three had only one risk factor, one had two, and four had four risk factors.

Data Analysis

Descriptive statistics are expressed as mean ± standard deviation (SD). Data were analyzed by analysis of variance (ANOVA). The groups were compared using two sample *t*-tests, and statistical significance was defined as $p < 0.05$. Values are presented as mean ± SD.

Results

Changes in Arterial Diameter

Results are expressed as percentage change in baseline values. In the 60 control subjects (Group 1), brachial arterial di-

ameter and blood flow increased by 19.1 ± 15.7% and 63.1 ± 90.4%, respectively, after forearm ischemia (Table I).

Subjects with risk factors for coronary artery disease (Group 2) showed a significantly attenuated brachial artery response compared with the control group. In this group, arterial diameter increased by 11.9 ± 95%, and blood flow by 43.0 ± 46.9% from baseline.

Patients with an acute ischemic syndrome (Group 3) showed virtually no change in brachial artery diameter or blood flow (1.2 ± 0.2%, and 0.2 ± 0.6%, respectively). Patients in Group 3 showed a statistically significant reduction in both arterial diameter and flow compared with those of Groups 1 and 2 ($p < 0.0001$) (Table I).

After the administration of nitroglycerin, brachial artery diameter and flow increased in all three groups, with no statistical significance between Groups 1 and 2 (arterial diameter: 26.3 ± 8.72% vs. 18.8 ± 12.1%, and arterial flow 74.4 ± 60.6% vs. 56.4 ± 16.2%). The increase in arterial diameter (10.1 ± 7.3%) and flow (18.4 ± 4.8%) during nitroglycerin administration in Group 3 was significantly smaller ($p < 0.001$ and $p < 0.005$, respectively) than in Groups 1 and 2 (Table I).

Brachial artery measurements were repeated 4 weeks after initial study in nine patients of Group 3 who remained totally asymptomatic after admission. These patients showed an increase in arterial diameter (11.1 ± 7.6%) and arterial flow (34.2 ± 38.9%) after forearm ischemia and after the administration of nitroglycerin (17.2 ± 9.7% and 61.3 ± 22.4%) that reached statistical significance with respect to controls (Group 1), values similar to those of subjects in Group 2 (Table I, Fig. 1).

TABLE I Results in post-ischemic brachial artery vasodilation response in the three groups

	Arterial diameter	Arterial flow	Arterial diameter (GNT)	Arterial flow (GNT)
Group 1 (%)	19.1 ± 15.7	63.1 ± 90.4	26.3 ± 8.72	74.4 ± 60.6
	↕	↕	↕	↕
	p 0.002	p 0.1	p 0.5	NS
	↕	↕	↕	↕
Group 2 (%)	11.9 ± 9.5	43.0 ± 46.9	18.8 ± 12.1	56.4 ± 16.2
	↕	↕	↕	↕
	p 0.0001	p 0.0001	p 0.001	p 0.005
	↕	↕	↕	↕
Group 3 (%)	1.2 ± 0.2	0.2 ± 0.6	10.1 ± 7.3	18.4 ± 14.8
	↕	↕	↕	↕
	p 0.0001	p 0.001	p 0.005	p 0.005
	↕	↕	↕	↕
Group 3 (%) (reassessed)	11.1 ± 7.6	34.2 ± 38.9	17.2 ± 9.7	61.3 ± 22.4

Group 1: healthy subjects with no cardiovascular risk factors. Group 2: healthy subjects with cardiovascular risk factors. Group 3: patients with unstable angina syndromes. Group 3 (reassessed): the nine patients from Group 3 reassessed 4 weeks after unstable angina. Results are expressed as mean ± SD of percentage change with respect to the basal values. Statistical p values are between arrows.

Abbreviations: GNT= glyceryl trinitrate-induced dilation, NS = not significant.

Discussion

Endothelial Dysfunction

In the present study, patients with unstable angina (Group 3) showed reduced brachial artery vasodilatory responses despite rapid clinical stabilization. The abnormal endothelial function in this group was more pronounced than that in the risk factor group (Group 2). This suggests the presence of an additional mechanism, probably associated with the inflammatory process underlying acute coronary syndromes. Our findings suggest that vasoactive and/or proinflammatory molecules generated or released at the site of disrupted plaques may contribute to the abnormal vasodilation. It is interesting to note that all nine patients in Group 3 who underwent retesting after an uneventful follow-up showed vasodilator responses comparable with those of the control subjects at 4 weeks.

Normal endothelium function results in the appropriate balance between vasodilator and vasoconstriction forces and appropriate vascular smooth muscle cell growth. Platelet adhesion and aggregation, as well as thrombogenic forces, are adequately antagonized. Many variables disrupt the normal function of the endothelium and may therefore alter vasomotor responses, thereby initiating a cascade of events that may lead to acute coronary syndromes. Inappropriate coronary vasoconstriction due to endothelial dysfunction develops in patients with high serum cholesterol,[11] smokers,[12–14] diabetics,[15] and hypertensives.[16–18] Old age[19,20] and menopause in women[21,22] are also associated with endothelial dysfunction. In our study, subjects in Group 2 showed an attenuation of normal postischemic vasodilation response in the brachial artery.

Our subjects in Group 2 were older than those in Group 1 (mean age 56.4 ± 14.6 vs. 44.8 ± 17.4, respectively), and this fact can account for the minor differences in the vasodilator re-

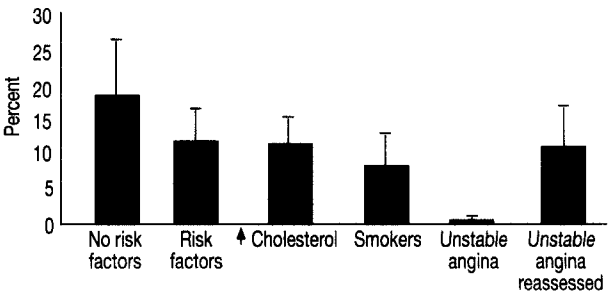

FIG. 1 Percentage of change of brachial artery vasodilation after ischemia in healthy subjects without cardiovascular risk factors (Group 1), healthy subjects with cardiovascular risk factors (Group 2), those with either hypercholesterolemia (cholesterol) or smokers, patients with unstable angina syndromes (Group 3), and patients with unstable angina syndrome reassessed 4 weeks later (unstable angina reassessed).

sponse observed in the study. However, patients in Group 3 were of a similar age to those in Group 2 (mean age 57.5 ± 5.6 vs. 56.4 ± 14.6), and it is therefore likely that the significant differences observed between Group 3 and the other two patient groups regarding endothelium-dependent vasodilation was due to causes other than age. Our findings in patients with risk factors are consistent with previous data in the literature.[11–23]

Previous studies have consistently demonstrated that patients with cardiovascular risk factors have an abnormal vasomotor response, and this is at least partially reversible with appropriate therapeutic intervention, that is, lipid-lowering treatment,[24–26] smoking cessation,[12–14] antioxidants,[27–29] and the administration of the NO precursor L-arginine.[30–32] It is interesting that our patients with unstable angina showed what has previously been termed as "endothelial stunning" or what perhaps may be considered to represent endothelial "hibernation." In these patients, endothelium function was found to be reduced on admission, and in some of those who were reassessed, endothelial function was comparable with normal controls a few weeks later. We could assume that medical treatment of cardiovascular risk factors after the unstable angina improved the endothelial function in these patients, but one of them had no risk factors and the improvement was impressive enough to support only this possibility.

Brachial Artery Studies

Assessment of coronary endothelial function would be desirable to investigate vasomotor responses in unstable angina directly. This, however, poses a series of problems. Invasive studies may not be suitable for the evaluation of patients who, like ours in Group 3, were totally asymptomatic following the initial therapeutic measures. Ultrasound measurements of brachial artery diameter may represent a surrogate. Changes as little as 0.1 mm can be measured reliably by high-resolution ultrasound imaging; these measurements have proved to be accurate and reproducible.[33] Maximum measurement differences among independent observers was ≤ 0.1 mm.[33] Previous studies have demonstrated that endothelial dysfunction is a generalized phenomenon detectable to a variable degree in a number of vascular beds in patients with cardiovascular risk factors.[6,7,19] Furthermore, it has been shown that the brachial artery response correlates closely with the coronary vasodilator response to acetylcholine.[8,9] There has been a good correlation between brachial and coronary artery responses within subjects.[8,9]

The present study also reopens the question of whether a single disrupted plaque is responsible for the generalized endothelial dysfunction, or whether generalized dysfunction facilitates plaque disruption.

Conclusions

The syndrome of unstable angina is associated with endothelial dysfunction that results in the abolition of postischemic vasodilation of the brachial artery. In patients who evolve uneventfully, endothelial function recovers after a few weeks. These findings suggest that both generalized but transient endothelial dysfunction develops in patients with rapidly stabilized unstable angina. Brachial ultrasound is a noninvasive test that may help to elucidate the role of endothelial dysfunction and the effects of therapeutic intervention in unstable angina.

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