Vascular Endothelial Function and Oxidative Stress Mechanisms in Patients With Behçet’s Syndrome

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OBJECTIVES We sought to test the hypothesis that vascular endothelial function is impaired in Behçet’s syndrome and reflects increased levels of oxidative stress.

BACKGROUND Behçet’s syndrome is a multisystem inflammatory disorder commonly complicated by vascular thrombosis and arterial aneurysm formation. The precise mechanisms underlying vascular disease in Behçet’s syndrome are not known.

METHODS We studied 19 patients with Behçet’s syndrome (18 to 50 years old, 9 men) and 21 healthy volunteers (18 to 50 years old, 10 men). Brachial artery flow-mediated dilation (endothelium-dependent), and nitroglycerin (NTG)-induced dilation (endothelium-independent) were measured. To investigate oxidative stress mechanisms, vascular studies were repeated 1 h after administration of vitamin C (1 g, intravenously) in 12 patients and 12 control subjects.

RESULTS Flow-mediated dilation was reduced in patients with Behçet’s syndrome as compared with control subjects (0.7 ± 0.9% vs. 5.7 ± 0.9%, p = 0.001). In contrast, there were no significant differences in the brachial artery diameter (4.2 ± 0.2 vs. 4.0 ± 0.2 mm, p = 0.47) or NTG-induced dilation (19.7 ± 1.9% vs. 19.7 ± 1.2%, p = 0.98). In regression analysis, Behçet’s syndrome was associated with impaired flow-mediated dilation independent of age, gender, brachial artery diameter, blood pressure, cholesterol and glucose. Vitamin C increased flow-mediated dilation in Behçet’s syndrome (0.2 ± 0.7% to 3.5 ± 1.0%, p = 0.002), but not in control subjects (4.3 ± 0.6% to 4.7 ± 0.4%, p = 0.51). In both groups, NTG-induced dilation and brachial artery diameter were unchanged after vitamin C treatment.

CONCLUSIONS Vascular endothelial function is impaired in Behçet’s syndrome and can be rapidly improved by vitamin C treatment. Our results support a role for oxidative stress in the pathophysiology of Behçet’s syndrome and provide a rationale for therapeutic studies aimed at reducing vascular complications in this disorder. (J Am Coll Cardiol 2001;37:517–20) © 2001 by the American College of Cardiology

Behçet’s syndrome is a chronic, multisystem disorder characterized by recurrent oral and genital ulceration, skin lesions and uveitis (1). In addition, ~25% of patients develop vascular complications, which may include superficial thrombophlebitis, deep vein and arterial thrombosis and arterial aneurysm formation (2).

The etiologic mechanisms underlying vascular disease in Behçet’s syndrome are not well understood. Histopathologic studies have demonstrated that the predominant lesion is vasculitis, affecting both the vessel wall and perivascular tissues (1). Reports of elevated serum concentrations of von Willebrand factor, plasminogen activator inhibitor-1 and thrombomodulin suggest the presence of vascular endothelial dysfunction in patients with Behçet’s, although these abnormalities have not been consistently found (3,4). Evidence from recent studies suggests that activated leukocytes may contribute to vascular injury in Behçet’s syndrome (5,6). Neutrophils from patients with Behçet’s syndrome generate high levels of oxygen-derived free radicals (5,6) and cause endothelial cell lysis in vitro (6). Concentrations of circulating pro-oxidants and lipid peroxidation products are elevated in patients with Behçet’s syndrome (7), although the relation between oxidative stress mechanisms and vascular injury in patients with Behçet’s syndrome has not been elucidated.

The purpose of the present study was to test the hypotheses: 1) that vascular endothelial function is impaired in Behçet’s syndrome; and 2) that endothelial dysfunction in Behçet’s syndrome is mediated by oxidative stress mechanisms and can be ameliorated by vitamin C treatment.

METHODS Subjects. We studied 19 patients with Behçet’s syndrome (18 to 50 years old, 9 men) and 21 healthy volunteers (18 to 50 years old, 10 men). Patients were identified at the Rheumatology Clinic at the Hammersmith Hospital; they had satisfied the International Study Group’s criteria for Behçet’s syndrome (Table 1) (8). All patients were considered to have active disease at the time of the study, on the basis of the presence of at least two of the major symptoms of Behçet’s syndrome. Thirteen patients were receiving treatment (prednisolone [n = 10], azathioprine [n = 6] and colchicine [n = 4]). Healthy volunteers were recruited from the hospital staff, and none were receiving drug treatment. All subjects were nonsmokers, with no history of diabetes, hypertension, hypercholesterolemia or atherosclerotic vascular disease. The study was approved by the local Ethics Committee, and all subjects gave informed, written consent.

Clinical history, including drug therapy, was recorded in...
Blood pressure was measured using a mercury sphygmomanometer, and height and weight were recorded. The subjects were studied after an overnight fast, and blood samples were collected for glucose, total cholesterol, high density lipoprotein (HDL) cholesterol and triglyceride levels (Olympus AU800 multichannel analyzer).

For each subject, brachial artery flow-mediated dilation (endothelium-dependent) and nitroglycerin (NTG)-induced dilation (endothelium-independent) were measured, as described subsequently. To investigate the role of oxidative stress mechanisms in the observed vascular responses, brachial artery measurements were repeated 1 h after administration of vitamin C (1 g in 100 ml of normal saline, intravenously over 30 min) in 12 patients and 12 control subjects.

Brachial artery vascular measurements. Brachial artery flow-mediated dilation was measured using a 7.0-MHz linear array transducer, an Acuson 128XP/10 system (Mountain View, California) and a high resolution ultrasonic vessel wall tracking system (Vadirec, Ingenious Systems, Netherlands). In brief, the brachial artery was scanned longitudinally, and the brachial artery diameter was measured using the wall tracking system. After the baseline rest scan, a pneumatic cuff placed at the level of the wrist was inflated to 300 mm Hg for 4.5 min. The second scan was performed 55 to 65 s after cuff deflation. Fifteen minutes were allowed for vessel recovery, after which the second baseline scan was performed. Nitroglycerin (400 μg) was then administered, and the fourth scan of the brachial artery was taken. The vessel diameter was measured by two independent observers who were unaware of the subjects’ clinical details and the type and stage of the study. The technique for measurement of brachial artery flow-mediated dilation is reproducible in our laboratory. The coefficient of variation for flow-mediated dilation is 2%, based on measurements taken from the same subjects, on separate days, under rest conditions. Flow-mediated dilation of the conduit arteries is endothelium-dependent and largely mediated by nitric oxide (9).

Data processing and statistical analysis. Data were analyzed using the Statistical Package for the Social Sciences, version 8.0. Continuous data are expressed as the mean value ± SEM. The independent samples t test was used to compare the mean values of flow-mediated dilation, as well as other variables, between the two study groups. Linear regression analyses were conducted to investigate the association of Behçet’s syndrome with flow-mediated dilation, independent of possible confounding effects. The effect of vitamin C on flow-mediated dilation, NTG-induced dilation and brachial artery diameter was studied in patients and control subjects separately, using the paired samples t test. Statistical significance was inferred at p < 0.05.

RESULTS

Clinical and biochemical characteristics. Compared with control subjects, patients with Behçet’s syndrome were similar in terms of age (40 ± 2 vs. 41 ± 2 years, p = 0.70),
body mass index (25 ± 1.4 vs. 25 ± 1.0 kg/m², p = 0.81),
diastolic blood pressure (71 ± 2 vs. 71 ± 2 mm Hg, p =
0.78), fasting glucose (5.3 ± 0.2 vs. 4.8 ± 0.1 mmol/liter,
p = 0.06), total cholesterol (5.2 ± 0.2 vs. 4.8 ± 0.1
mmol/liter, p = 0.76) and HDL cholesterol (1.5 ± 0.1 vs.
1.5 ± 0.1 mmol/liter, p = 0.80). Systolic blood pressure was
higher in patients than in control subjects (123 ± 3 vs.
112 ± 3 mm Hg, p = 0.02).

**Brachial artery flow-mediated dilation.** Flow-mediated,
endothelium-dependent dilation was reduced in patients
with Behçet’s syndrome as compared with control subjects
(0.7 ± 0.9 vs. 5.7 ± 0.9%, respectively, p = 0.001) (Fig. 1).
In contrast, there were no significant differences in baseline
brachial artery diameter (4.2 ± 0.2 vs. 4.0 ± 0.2 mm, p =
0.47) or in brachial artery dilation in response to sublingual
NTG (19.7 ± 1.9 vs. 19.7 ± 1.2%, p = 0.98) between
patients with Behçet’s syndrome and control subjects. On
regression analysis, Behçet’s syndrome was associated with
impaired flow-mediated dilation (partial regression coeffi-
cient −4.9 ± 1.9%, p = 0.01), independent of age, gender,
blood pressure, total and HDL cholesterol, triglycerides,
glucose and baseline brachial artery diameter.

**Effects of vitamin C on vascular responses.** In patients
with Behçet’s syndrome (n = 12), administration of vitamin
C was associated with an increase in flow-mediated dilation
at 1 h (0.2 ± 0.7 to 3.5 ± 1.0%, p = 0.002) (Fig. 2). In
contrast, NTG-induced dilation and baseline brachial artery
diameter were unchanged after vitamin C treatment (16.9 ±
Dilation (18.6 ± 1.9 vs. 14.8 ± 1.3%, \( p = 0.26 \); 4.1 ± 0.2 vs. 4.2 ± 0.3 mm, \( p = 0.99 \)). In control subjects, administration of vitamin C had no significant effect on flow-mediated dilation (4.3 ± 0.6 to 4.7 ± 0.4%, \( p = 0.51 \)) (Fig. 2), NTG-induced dilation (18.6 ± 1.1 vs. 21.0 ± 2.2%, \( p = 0.41 \)) or brachial artery diameter (4.0 ± 0.2 vs. 4.0 ± 0.2 mm, \( p = 0.66 \)).

DISCUSSION

We have found that vascular endothelial function is impaired in patients with Behcet's syndrome and can be rapidly improved by treatment with the antioxidant vitamin C. Our findings of endothelial dysfunction—mediated by increased oxidative stress—provide novel insight into the mechanisms underlying the vascular manifestations of Behcet's syndrome.

Endothelial function is impaired in Behcet's syndrome. Previous studies investigating endothelial function in patients with Behcet's syndrome have not been consistent, although they used indirect markers of vascular injury (3,4). In the present study, we found that brachial artery flow-mediated dilation is impaired in patients with Behcet's syndrome, as compared with healthy control subjects. Because flow-mediated dilation is endothelium-dependent and mediated largely by the release of endothelial nitric oxide (9), our findings imply that endothelial nitric oxide activity is impaired in Behcet's syndrome. Our observations are consistent with previous reports of reduced plasma nitrates and nitrites in patients with active Behcet's syndrome (10) and may help explain the vascular manifestations of this disorder. Reduced activity of nitric oxide, the major endothelium-derived vasodilator, may lead to vasoconstriction, platelet aggregation and monocyte adhesion, which separately or together may promote vascular disease in patients with Behcet's syndrome.

Mechanisms underlying endothelial dysfunction in Behcet's syndrome. The mechanisms underlying endothelial dysfunction in Behcet's syndrome are not known. In the present study, vitamin C, an antioxidant that scavenges superoxide anion radicals, rapidly increased flow-mediated dilation in patients with Behcet's syndrome. Our results suggest that endothelial dysfunction is mediated by increased oxidative stress and are consistent with previous observations of elevated levels of lipid peroxides, as well as increased neutrophil production of superoxide, in patients with Behcet's syndrome (5,7). Oxidative stress is a key factor in vascular injury. Previous studies have shown that oxygen-derived free radicals, including superoxide anion, react with nitric oxide, thus reducing its availability (11). However, our results do not exclude an additional role for inflammatory cytokines, anti-endothelial cell antibodies or vasoconstrictors, such as asymmetric dimethyl arginine (12–14), underlying endothelial dysfunction in Behcet's syndrome.

Clinical implications of our results. Currently there are no reliable means to identify patients with Behcet's syndrome who have increased susceptibility to future vascular complications. Measurement of brachial artery flow-mediated dilation may provide a simple, reproducible and noninvasive technique to identify patients at increased risk of vascular disease. In addition, our observations—that vascular endothelial function is impaired in Behcet's syndrome, can be improved by vitamin C treatment and may be mediated by increased oxidative stress—provide a rationale for the use of antioxidant vitamins to reduce vascular complications in this disorder. However, because the short-term effects of vitamin C treatment have not always been maintained (15), a long-term intervention trial will be needed to formally evaluate this possibility.

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