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Relationship between nitroglycerine-induced vasodilation and clinical severity of peripheral artery disease

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ABSTRACT

Objective: Nitroglycerine-induced vasodilation is usually used as a control test for flow-mediated vasodilation (FMD). However, nitroglycerine-induced vasodilation per se has also been reported to be impaired in patients with atherosclerosis. The purpose of this study was to determine the relationship between nitroglycerine-induced vasodilation and the clinical severity of peripheral artery disease (PAD).
Methods and results: We measured nitroglycerine-induced vasodilation and FMD in 144 subjects (mean age: 63.8 ± 15.1 years), including 32 PAD patients with critical limb ischemia (CLI group), 28 PAD patients without CLI (non-CLI group), 60 age- and sex-matched patients without established cardiovascular disease (at-risk group), and 24 healthy subjects (healthy group). Nitroglycerine-induced vasodilation was significantly impaired in the CLI group compared to that in the other three groups (healthy group, 16.0 ± 5.3%; at-risk group, 12.9 ± 3.8%; non-CLI group, 10.3 ± 5.1%; CLI group, 6.7 ± 3.9%; $P < 0.05$, respectively). Even after multivariate adjustment, the differences remained significant. On the other hand, FMD was significantly impaired in the at-risk, non-CLI, and CLI group compared with that in the healthy group (healthy group, 7.1 ± 2.9%; at-risk group, 3.4 ± 2.3%; non-CLI group, 3.5 ± 2.7%; CLI group, 3.0 ± 2.8%; $P < 0.001$, respectively), but the differences among the at-risk, non-CLI, and CLI groups were not significant. Multivariate analysis revealed that nitroglycerine-induced vasodilation (odds ratio: 0.77, 95% confidence interval [CI]: 0.61–0.97) and diabetes mellitus (odds ratio: 8.75, 95% CI: 1.74–44.2) were independent variables for CLI in PAD patients.
Conclusions: There was no significant difference in FMD between PAD patients with and those without CLI, but nitroglycerine-induced vasodilation was significantly smaller in PAD patients with CLI compared with those without CLI.

1. Introduction

Patients with peripheral artery disease (PAD) are at markedly increased risk for cardiovascular events due to their multiple atherosclerosis risk factors and extensive atherosclerotic disease [1,2]. Critical limb ischemia (CLI) is the most advanced form of PAD and is characterized by chronic ischemic rest pain, ischemic ulcers or gangrene [3–5]. Natural history studies have revealed that less than 10% of PAD patients with claudication progress to CLI [6–8].

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However, compared to patients with intermittent claudication, patients with CLI have been reported to have a very high risk for fatal and non-fatal vascular events and for limb amputation, with a one-year mortality rate of about 25% and a six-month amputation rate of about 25% [3].

Nitroglycerine-induced vasodilation, a vascular response to a sublingually administered nitroglycerine tablet in the brachial artery, is usually measured for the assessment of endothelium-independent vasodilation as a control test for flow-mediated vasodilation to assure that vascular response to hyperemia is not influenced by underlying vascular smooth muscle dysfunction or alterations of vascular structure but is truly a consequence of endothelial function [9,10]. Recently, it has been reported that nitroglycerine-induced vasodilation per se is impaired in patients with multiple cardiovascular risk factors or established cardiovascular disease [11–14]. As for the relationship between PAD and vascular function, it is known that PAD is associated with endothelial dysfunction [15,16]. However, there is little information on the relationship between nitroglycerine-induced vasodilation and PAD. In addition, difference in vascular function between PAD patients with and those without CLI remains unknown. We therefore measured nitroglycerine-induced vasodilation and FMD in PAD patients with and those without CLI to assess vascular function and to determine the association between vascular function and the clinical severity of PAD.

2. Methods

2.1. Subjects

A total of 144 subjects, including 32 PAD patients with CLI (CLI group: 23 men and 9 women, mean age: 67.6 ± 7.6 years), 28 PAD patients without CLI (non-CLI group: 22 men and 6 women, mean age: 69.8 ± 8.7 years), 60 age- and sex-matched patients with cardiovascular risk factors but without established cardiovascular disease (at-risk group: 45 men and 15 women, mean age: 68.7 ± 8.0 years) and 24 healthy control subjects (healthy group: 10 men and 14 women, mean age: 39.3 ± 18.0 years), were enrolled. PAD was defined as current intermittent claudication with ABI <0.9, or chronic ischemic rest pain, ischemic ulcers, or gangrene attributed to objectively proven arterial occlusive disease, or history of previous intervention, including angioplasty, bypass graft, and limb amputation. Diagnosis of limb ischemia was confirmed by angiography. CLI was classified according to the guidelines of Trans Atlantic Inter-Society Consensus II [3]. Healthy control subjects had no history of cardiovascular diseases, liver diseases, renal diseases, autoimmune diseases, or malignant diseases and had no coronary risk factors, including hypertension, dyslipidemia, diabetes mellitus, and smoking. Hypertension was defined as treatment with oral antihypertensive agents or systolic blood pressure of more than 140 mm Hg or diastolic blood pressure of more than 90 mm Hg, in a sitting position, on at least 3 different occasions without medication [17]. Diabetes was defined according to the American Diabetes Association recommendation [18]. Dyslipidemia was defined according to the third report of the National Cholesterol Education Program [19]. We defined smokers as those who had ever smoked. One pack-year was equivalent to 20 cigarettes per day for 1 year. Coronary heart disease (CAD) included angina pectoris, myocardial infarction, and unstable angina. Cerebrovascular disease (CVD) included ischemic stroke, hemorrhagic stroke, and transient ischemic attack. The vascular tests were performed without withholding medications. The ethical committees of our institutions approved the study protocol. Written informed consent for participation in the study was obtained from all subjects.

2.2. Study protocol

We measured vascular responses to reactive hyperemia and sublingually administrated nitroglycerine in the brachial artery in all subjects. Subjects fasted the previous night for at least 12 h. The study began at 8:30 AM. The subjects were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature of 22 °C–25 °C) throughout the study. A 23-gauge polyethylene catheter was inserted into the left deep antecubital vein to obtain blood samples. Thirty minutes after maintaining the supine position, basal brachial artery diameter was measured. Then FMD was measured. After completion, we next measured nitroglycerine-induced vasodilation with confirmation that the brachial artery diameter had recovered to the baseline value. The observers were blind to the form of examination.

2.3. Measurement of FMD and nitroglycerine-induced vasodilation

Vascular response to reactive hyperemia in the brachial artery was used for assessment of endothelium-dependent FMD. A high-resolution linear artery transducer was coupled to computer-assisted analysis software (UNEXEF18G, UNEX Co, Nagoya, Japan) that used an automated edge detection system for measurement of brachial artery diameter. A blood pressure cuff was placed around the forearm. The brachial artery was scanned longitudinally 5–10 cm above the elbow. When the clearest B-mode image of the anterior and posterior intimal interfaces between the lumen and vessel wall was obtained, the transducer was held at the same point throughout the scan by a special probe holder (UNEX Co) to ensure consistency of the image. Depth and gain setting were set to optimize the images of the arterial lumen wall interface. When the tracking gate was placed on the intima, the artery diameter was automatically tracked, and the waveform of diameter changes over the cardiac cycle was displayed in real time using the FMD mode of the tracking system. This allowed the ultrasound images to be optimized at the start of the scan and the transducer position to be adjusted immediately for optimal tracking performance throughout the scan. Pulsed Doppler flow was assessed at baseline and during peak hyperemic flow, which was confirmed to occur within 15 s after cuff deflation. Blood flow velocity was calculated from the color Doppler data and was displayed as a waveform in real time. The baseline longitudinal image of the artery was acquired for 30 s, and then the blood pressure cuff was inflated to 50 mm Hg above systolic pressure for 5 min. The longitudinal image of the artery was recorded continuously until 5 min after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 s at baseline and for 10 s immediately after cuff deflation. Changes in brachial artery diameter were immediately expressed as percentage change relative to the vessel diameter before cuff inflation. FMD was automatically calculated as the percentage change in peak vessel diameter from the baseline value. Percentage of FMD [(Peak diameter-Baseline diameter)/Baseline diameter] was used for analysis. Blood flow volume was calculated by multiplying the Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area (πr^2). Reactive hyperemia was calculated as the maximum percentage increase in flow after cuff deflation compared with baseline flow.

The response to nitroglycerine was used for assessment of endothelium-independent vasodilation. Nitroglycerine-induced vasodilation was measured as described previously [14]. Briefly, after acquiring baseline rest images for 30 s, a sublingual tablet (75 μg nitroglycerine) was given, and images of the artery were recorded continuously until the dilation reached a plateau after administration of nitroglycerine. Subjects who had received nitrate treatment and subjects in whom the sublingually administered