Role of Endothelial Nitric Oxide in Shear Stress–Induced Vasodilation of Human Microvasculature

Diminished Activity in Hypertensive and Hypercholesterolemic Patients

Oscar A. Paniagua, MD; Melissa B. Bryant, RN; Julio A. Panza, MD

Background—It has been proposed that flow-mediated shear stress regulates vascular tone; however, whether this operates in the human microcirculation is unknown. This study was designed to investigate the effect of shear stress on human microvascular tone, to assess the contribution of nitric oxide (NO), and to determine whether this mechanism is defective in hypertension and in hypercholesterolemia.

Methods and Results—In 9 normal controls (NC), 11 hypertensive patients (HT), and 12 hypercholesterolemic patients (HChol), arteries (internal diameter 201±26 μm) isolated from gluteal fat biopsies were cannulated and perfused in chambers. Shear stress was induced by increasing the flow rate from 1 to 50 μL/min after preconstriction with norepinephrine (NE). Arterial internal diameter was expressed as percent of NE-induced constriction. In NC, shear stress induced flow-dependent vasodilation from 23±9% at 1 μL/min to 53±14% at 50 μL/min (P<0.0001), which was abolished by endothelial removal. The NO synthase inhibitor Nω-nitro-l-arginine (L-NNA) significantly blunted this response (mean vasodilation decreased from 27±6% to 6±9%; P=0.04). HT had significant impairment of flow-mediated dilation (mean vasodilation 5±6%; P=0.01 versus NC), which was not affected by L-NNA. HChol had preserved flow-mediated vasodilation (mean vasodilation 24±7%; P=0.56 versus NC), but this was not significantly modified by L-NNA.

Conclusions—In the human microvasculature, shear stress induces endothelium-dependent, NO-mediated vasodilation. This phenomenon is blunted in HT patients because of reduced activity of NO. In contrast, the HChol microvasculature has preserved shear stress-induced dilation despite diminished NO activity. (Circulation. 2001;103:1752-1758.)

Key Words: microcirculation, endothelium, nitric oxide, hypertension, hypercholesterolemia

Endothelial cells control vascular tone through the release of different factors that determine the contractile activity of the underlying smooth muscle.1 This regulatory function of the endothelium can be modulated by endogenous substances (eg, bradykinin, serotonin), pharmacological agents (eg, acetylcholine, substance P), and mechanical forces such as flow-mediated shear stress. Among these, shear stress is probably the most relevant physiological stimulation for the release of endothelial factors and thereby for the maintenance of normal vascular tone.2 In fact, previous studies in animal models have shown that shear stress stimulates the release of vasoactive factors by the microvascular endothelium.3–5 In humans, previous studies have shown that shear stress induces endothelium-dependent vasodilation of conductance vessels, which is impaired in atherosclerosis, both in the coronary6,7 and peripheral8 circulations. This flow-mediated vasodilation is due in part to the release of nitric oxide (NO).9 However, whether flow-mediated shear stress modulates NO activity and consequently vascular tone in human resistance vessels has not been investigated previously.

Patients with essential hypertension10,11 and patients with hypercholesterolemia12,13 have impaired endothelium-dependent vasodilation of the microcirculation in response to pharmacological agents. This abnormality is related to decreased NO activity14,15 and, in the case of hypertensive patients, may contribute to their increased systemic vascular resistance and impaired vasodilation in response to mental stress.16 However, whether this defect affects the physiological control of vascular tone by shear stress has not been determined.

Therefore, the present study was designed to investigate the effect of changes in shear stress on microvascular tone in normal humans, in patients with essential hypertension, and in patients with hypercholesterolemia. We hypothesized that shear stress modulates vascular tone in human resistance arteries, that this phenomenon is mediated by NO, and that this mechanism is defective in hypertension and in hypercholesterolemia.

Methods

Study Population

The clinical characteristics of the study population are reported in the Table. Before admission into the study, subjects of each group were screened by clinical history, physical examination, routine chemical analyses, electrocardiography, and chest radiograph. Exclusion cri-
Characteristics of the Study Population

<table>
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<tr>
<td>Triglycerides, mg/dL†</td>
<td>92±68</td>
<td>84±30</td>
<td>163±108</td>
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</table>

Data are mean±SD.

*P<0.001 vs normal controls by 1-way ANOVA with Bonferroni correction.
†P=0.04 for difference among the 3 groups by 1-way ANOVA; the Bonferroni t test did not detect statistically significant differences in the multiple comparisons of patients vs normal controls.

Subcutaneous Biopsies and In Vitro Procedures

In each subject, a biopsy sample of skin and subcutaneous tissue (~0.5 cm wide×1.2 cm long×1.5 cm deep) was taken from the gluteal region under local anesthesia. The specimen was immediately placed in cold physiological saline solution (PSS) and transported to a laboratory where subcutaneous arterioles (internal diameter 201±26 μm) were dissected under a light microscope. After removal of surrounding adipose and connective tissue, a segment of the artery (length ~3 mm) was transferred to a 15-ml vessel chamber (Living System Instrumentation) containing cold PSS and 2 glass microcannulas. The proximal end of the artery was slipped onto the proximal cannula and secured with microsurgery thread. The residual blood in the artery was gently flushed, and the distal end was then slipped onto the distal cannula and similarly secured. Approximately 2 mm of the arterial segment lay between the cannulas. The axial length of the vessel segment was set by carefully modifying the position of the proximal cannula to eliminate warping or buckling and avoiding excessive longitudinal stretch. Pressure transducers were connected proximally and distally to measure intravascular pressure and the pressure gradient for a given flow rate during the experiments.

Once the artery was mounted, the chamber was transferred to the stage of an inverted microscope and connected to a reservoir containing PSS with the following composition (in mmol/L): 119.0 NaCl, 4.7 KCl, 1.76 CaCl₂, 1.17 MgSO₄, 5.5 glucose, 17 NaHCO₃, 1.17 KH₂PO₄, and 0.026 K-EDTA. From the reservoir, the vessel chamber was continuously suffused at a rate of 40 mL/min with PSS equilibrated with a gas mixture of 95% O₂ and 5% CO₂. The pH of the PSS was 7.4, and the temperature was maintained at 37°C with a water thermal regulator. Vessels were checked to ascertain the absence of leaks by determining their ability to maintain a given intravascular pressure. Vessels were then left for 1 hour under no-flow conditions for equilibration.

Experimental Protocol

After the equilibration period, vessels were exposed to PSS with high K⁺ content (composition in mmol/L: 78.6 NaCl, 60 KCl, 2.5 CaCl₂, 1.17 MgSO₄, 17 NaHCO₃, 1.17 KH₂PO₄, 5.5 glucose, and 0.026 K-EDTA). Arteries that did not constrict to <50% of the basal internal diameter were considered not viable and discarded.

The proximal cannula was connected to a microinfusion syringe pump (Harvard Apparatus) used to modify the intraluminal flow rate. After preconstriction with a submaximal dose of norepinephrine (NE; 10⁻⁵ mol/L), basal flow-mediated dilation was determined by increasing intraluminal flow rate in a stepwise fashion from 1 to 2, 5, 10, 15, 20, 25, 30, 35, 40, and 50 μL/min. Flow was maintained for at least 3 minutes at each flow rate, allowing the vessel to reach a stable diameter for each stage. Using the pressure servo system, distal pressure was reduced by 50% of the pressure gradient generated at each flow rate to maintain a constant intraluminal pressure of 80 mm Hg throughout the study.

After washout, each artery was incubated with Nω-nitro-L-arginine (L-NNA; 10⁻⁴ mol/L) for 30 minutes. Subsequently, NE-induced contraction and flow-mediated dilation were repeated by procedures identical to those described above.

To determine whether flow-mediated dilation depended on the presence of the endothelium, similar experiments were performed in separate vessels from 5 normal controls in which the endothelium was removed by the introduction of an air bubble into the vessel lumen. In these vessels, and after the flow experiments were completed, successful endothelium removal was ascertained by the lack of vasodilator response to acetylcholine (10⁻⁶ mol/L) and preserved response to sodium nitroprusside (SNP; 10⁻⁴ mol/L). To ascertain that any impairment in flow-mediated dilation was not due to decreased responsiveness of the vascular smooth muscle, endothelium-independent responses to SNP were assessed in separate blood vessels isolated from the same biopsy sample. In these vessels, after preconstriction with NE (10⁻⁵ mol/L), dose-response
curves to SNP (10⁻⁹ to 10⁻⁷ mol/L) were obtained under no-flow conditions while an intraluminal pressure of 80 mm Hg was maintained.

Analysis of Vascular Responses
Vascular responses were assessed by measurement of the internal diameter of the arterial segment at the end of each stage of the experimental protocol. To this end, images of the blood vessel were continuously captured by a video camera mounted on an inverted microscope and projected on a TV monitor. After the vessel reached a stable diameter at each stage of the protocol (usually within 2 to 3 minutes), 10 seconds of in vivo images displayed on the monitor were recorded on a VHS videotape. Images were subsequently digitized and analyzed offline by a commercially available system (Eastman Kodak). The internal diameter of the arterial segment was measured with electronic calipers. If vasomotion was present, the average of the maximum and minimum diameters was used for calculations.

Statistical Analysis
Data are presented as mean±SEM, except where indicated. For each flow-mediated dilation or dose-response curve, data are expressed as percent of the NE-induced constriction for each vessel using this formula: vascular response (%)=([current diameter−diameter after NE]/diameter before NE]×100. Thus, 0% represents no change with respect to the diameter measured after NE-induced constriction, and 100% represents maximal vasodilation with complete return of the vessel diameter to the value measured before constriction.

Group differences were analyzed by 1-way ANOVA with Bonferroni correction for multiple comparisons versus the control group and by χ² test, as appropriate. Flow-dependent dilation and dose-response curves were compared with 2-way ANOVA for repeated measurements. The Student-Newman-Keuls method was used for pairwise multiple comparisons. A P value <0.05 was considered to indicate statistical significance.

Results
Shear Stress–Induced Vasodilation of the Normal Human Microvasculature
In arteries from normal controls, flow rate–dependent shear stress induced progressive vasodilation from 23±9% at 1 µL/min to a maximum of 53±14% at 50 µL/min (P<0.001). In the subset of 5 vessels in which the endothelium was removed, there was virtual abolition of flow-mediated vasodilation (mean vasodilation before and after endothelium removal: 25±5% versus 3±6%, respectively; P<0.001) (Figure 1). NO synthesis inhibition with L-NNA significantly blunted the response to increases in flow (mean vasodilation before and after L-NNA: 27±6% versus 6±9%, respectively; P=0.04) (Figure 2).

Shear Stress–Induced Vasodilation of Hypertensive and Hypercholesterolemic Microvessels
Arteries from hypertensive patients had significant impairment of flow-mediated dilation compared with those from normal controls (Figure 3). Mean vasodilation was 5±7% versus 27±6%, respectively (P=0.01), and maximal vasodilation at 50 µL/min was 17±10% versus 53±14%, respectively (P=0.04). NO synthase inhibition with L-NNA did not significantly affect shear stress–induced dilation in arteries from hypertensive patients (mean vasodilation before and after L-NNA: 5±7% versus 12±5%, respectively; P=0.11) (Figure 4).

Arteries from hypercholesterolemic patients had preserved flow-mediated vasodilation compared with those from normal controls (Figure 3). Mean vasodilation was 24±7% and 27±6%, respectively (P=0.56), and maximal vasodilation was 41±9% and 53±14%, respectively (P=0.44). However, in contrast to the response observed in vessels from normal controls, incubation with L-NNA did not significantly affect shear stress–induced dilation of hypercholesterolemic arteries (mean vasodilation before and after L-NNA: 24±7% versus 18±6%, respectively; P=0.50) (Figure 5).

The vasodilator response to SNP was not significantly different among the 3 groups (mean vasodilation in normal controls, hypertensive, and hypercholesterolemic patients was 33±7%, 28±5%, and 34±6%, respectively; P=0.60) (Figure 6).

Discussion
Flow-Dependent Dilation of Human Microvessels
The present study results demonstrate that flow-mediated, shear stress–induced vasodilation is operative in the normal
human microvasculature. Thus, when arterioles of ~200-μm
diameter taken from normal humans were subjected to
progressive increases in flow, an increase in lumen diameter
was apparent that was directly related to flow rate. This
response is dependent on the presence and integrity of the
microvascular endothelium, because when the endothelial
lining was removed, the flow-dependent vasodilation was
abrogated.

Previous studies have shown that increases in blood flow
induce endothelium-dependent dilation of large conductance
arteries. The findings of the present study provide direct
evidence that flow-mediated increases in shear stress also
induce endothelium-dependent vasodilation of the human
microvasculature. Because these vessels determine vascular
resistance and therefore modulate blood flow, this phenom-
enon may have physiological relevance for the regulation of
regional perfusion and the maintenance of systemic vascular
tone.

Because endothelial regulation of vascular tone involves
the release of several factors, it is important to determine
which of them mediates a particular response. NO is contin-
uously synthesized and released by endothelial cells to
produce vasodilation by stimulating guanylate cyclase in the
underlying smooth muscle. Given the significance of NO
for the regulation of vascular tone, we hypothesized that this
molecule is also responsible for flow-mediated vasodilation
of the human microvasculature. Our observations indicate
that NO is indeed largely responsible for the shear stress–
induced dilation of microvessels. When vessels were prein-
cubated with L-NNA (an antagonist of NO synthesis), flow-
mediated dilation was substantially blunted, in fact to an
extent not too dissimilar to that observed with endothelium
removal. These findings are consistent with those observed in
animal microvessels and in large conductance human arteries in vivo.

Flow-Dependent Microvascular Dilation in
Hypertensive Patients
The present study demonstrates that flow-dependent dilation
of resistance arteries is impaired in hypertensive patients.
Furthermore, in our study, flow-mediated dilation of hypertensive arteries was not significantly modified by NO synthesis inhibition, indicating that NO activity in response to shear stress is reduced in hypertension and may be responsible for the diminished responses to increases in flow. Importantly, this is not due to a diminished vascular smooth muscle response to NO, because in the same vessels, SNP (a direct NO donor) induced a normal degree of vasodilation.

Previous in vitro studies of the hypertensive microvasculature have revealed morphological changes in the vascular wall and functional abnormalities in response to pharmacological stimulation. In vivo studies have identified impaired endothelium-dependent vasodilation in hypertensive patients due to reduced NO bioactivity. The results of the present study are concordant with those observations and provide new evidence that such a defect not only affects the response to pharmacological agonists (as shown previously) but, perhaps more importantly, limits the blood vessel response to shear stress, a physiological mechanism participating in the regulation of microvascular tone. Studies in animal models of hypertension have also shown impaired flow-dependent dilation of small arteries. In those animal models, endothelial NO activity in response to shear stress has been shown to be impaired or preserved, depending on the vascular territory under investigation.

A depressed shear stress–dependent release of NO may be responsible for the development and/or maintenance of increased peripheral resistance in hypertension. Thus, the loss (or significant impairment) of NO-mediated shear stress–induced vasodilation could in itself depress vascular relaxation in response to a variety of other stimuli, such as exercise, and thereby increase (or prevent the decrease of) vascular resistance, leading to elevated blood pressure. Moreover, a defective flow-mediated dilation may also be responsible for structural changes leading to the increased vascular resistance observed in established hypertension. In fact, reduction in blood flow in animals has been linked to the pathophysiology of hypertension by enhancing smooth mus-
cle cell mitogenesis and vascular hypertrophy via mechanisms that impair the inhibitory effects of NO on smooth muscle proliferation.25

Flow-Dependent Microvascular Dilation in Hypercholesterolemic Patients

In the present study, resistance arteries from hypercholesterolemic patients showed preserved flow-mediated vasodilation. However, NO synthesis inhibition with L-NNA did not significantly modify their shear stress–induced vasodilation, in contradistinction to the observed effects of the arginine analogue on normal vessels. The vasodilator response to SNP in hypercholesterolemic arteries was similar to that of normal vessels.

The finding of preserved flow-mediated endothelium-dependent vasodilation of the hypercholesterolemic microvasculature differs from the results of previous studies from our and other laboratories showing impaired endothelium-dependent vasodilation of resistance vessels in hypercholesterolemic patients.12,13,15 This discrepancy may be explained by differences in the mechanisms leading to endothelium-dependent vasodilation between pharmacological and physical stimulation. In fact, previous studies from our laboratory suggested a selective defect of endothelial vasodilator function in the microvasculature of these patients.26 The present study results complement those observations by demonstrating that the microvascular response to a physiological stimulus, such as shear stress, is preserved in hypercholesterolemic individuals.

Our results differ from previous reports of impaired flow-mediated dilation of large conductance arteries from hypercholesterolemic patients.27 This difference most likely reflects the behavior of the vascular beds under investigation. In fact, it is not surprising that flow-mediated dilation of the microvasculature is preserved in normotensive hypercholesterolemic subjects, such as those included in the present study. Thus, if shear stress–induced vasodilation of human microvessels is important for the physiological regulation of their vascular tone, then an impairment of this mechanism would lead to an increase in systemic vascular resistance and consequently elevated blood pressure. Therefore, the exclusion of hypercholesterolemic patients with high blood pressure from the present investigation may have prevented us from observing impairment in flow-mediated vasodilation of the microvasculature secondary to hypercholesterolemia.

Of note, the preserved shear stress–induced dilation of hypercholesterolemic microvessels was not significantly modified by NO synthesis inhibition, thus indicating a diminished role of NO. It is reasonable to speculate that increased activity of other (ie, non-NO) endothelial vasodilator factors, such as endothelium-derived hyperpolarizing factor (EDHF) and/or prostacyclin, takes a more prominent role and accounts for vascular tone homeostasis in the context of diminished NO activity. In fact, previous studies have suggested that during NO inhibition, EDHF has an increased role in the endothelium-dependent microvascular response to bradykinin.28

Conclusions

The present investigation demonstrates that shear stress secondary to increases in flow induces endothelium-dependent NO-mediated vasodilation of the normal human microvasculature. This response is blunted in microvessels from hypertensive patients owing to reduced activity of endothelial NO. In contrast, the hypercholesterolemic microvasculature has preserved shear stress–induced dilation in spite of diminished NO activity, presumably because of an increased role of other endothelial vasoactive factors. These findings extend previous observations of impaired endothelial microvascular responses to pharmacological agonists to a more physiologically relevant stimulus for the regulation of vascular tone. At the same time, they emphasize the differences in the mechanisms leading to the vascular abnormalities characteristic of hypertensive and hypercholesterolemic patients.

References

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