Endothelin-1 and Vascular Tone in Subjects With Atherogenic Risk Factors

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Abstract—Endothelin-1 (ET-1) is a potent vasoconstrictor that increases vascular tone in the resistance vessels of subjects with hypertension. It is unclear whether endogenous ET-1 affects resistance-vessel function equally in patients with other cardiovascular risk factors. Vasoconstriction to ET-1 is mediated principally via the endothelin-A (ET_A) receptor on vascular smooth muscle cells. Accordingly, we used an ET_A-specific antagonist, BQ-123, to test the hypothesis that endogenous ET-1 increases vascular resistance selectively in subjects with hypertension compared with other risk factors. BQ-123 was infused at 100 nmol/min for 80 minutes into the brachial artery of 10 subjects with hypertension (mean±SEM arterial pressure, 106±5 mm Hg), 12 subjects with hypercholesterolemia (mean±SEM total cholesterol, 7.1±0.2 mmol/L), 10 active smokers (mean±SEM, 42±11 pack-years), and 11 healthy, age-matched individuals. Forearm blood flow (FBF) was measured by venous occlusion plethysmography. BQ-123 dilated resistance arterioles in hypertensive subjects, with FBF’s increasing by 46±7% from baseline (P<0.001). BQ-123 increased FBF to a lesser extent in hypercholesterolemic (24±5%, P<0.001) and healthy (20±8%, P=0.007) individuals but did not affect FBF significantly in smokers (10±8%, P=0.185). The vasodilator response in hypertensive subjects, but not in hypercholesterolemic patients or smokers, was significantly greater than that in healthy individuals (P<0.012). Endogenous ET-1, acting via the ET_A receptor, increases resistance-vessel tone in subjects with hypertension more than in subjects with hypercholesterolemia or in smokers. These results indicate that ET-1 contributes more to the pathophysiology of hypertension than of other risk factors in subjects without overt atherosclerosis. (Hypertension. 2003;42:43-48.)

Key Words: endothelin ■ endothelium ■ risk factors ■ hypertension, chronic ■ hypercholesterolemia ■ smoking

Endothelin-1 (ET-1), a potent, endogenous vasoconstrictor, is released abuminally by endothelial cells and exerts its effects through the activation of endothelin-A (ET_A) and ET_B receptors. ET_A receptors are present on vascular smooth muscle cells, whereas ET_B receptors are located predominantly on endothelial cells. ET_A receptor activation results in vasoconstriction and smooth muscle proliferation, whereas ET_B receptor binding stimulates nitric oxide (NO) synthesis, resulting in vasodilation. ET_B receptors are also present on vascular smooth muscle cells and mediate vasoconstriction.

ET-1 levels are elevated in patients with atherosclerosis and atherosclerotic coronary lesions exhibit enhanced ET-1–mediated vascular tone. Risk factors for atherosclerosis are also associated with elevated plasma ET-1 levels. Several studies have found that endogenous ET-1 causes vasoconstriction in the resistance vessels of subjects with hypertension. One group has reported that ET-1 also contributes to vascular tone in subjects with hypercholesterolemia, and there are conflicting reports regarding the response to ET-1 blockade in diabetic individuals. There have been no studies evaluating the role of endogenous ET-1 in cigarette smokers. There has also been a difference of opinion regarding the effect of ET-1 on vascular resistance in healthy individuals. The fact that these observations come from several different laboratories makes it difficult to gauge whether ET-1 contributes to vascular tone equally among the various cardiac risk factors.

To evaluate the relative contribution of ET-1 to vasomotor dysfunction in patients with atherogenic risk factors, we performed a single, comparative study with an ET_A-selective antagonist, BQ-123 (D-Trp-D-Asp-Pro-d-Val-Leu), to compare the activity of endogenous ET-1 in the forearm resistance vessels of subjects with hypertension, hypercholesterolemia, and cigarette smokers, relative to healthy volunteers.

Methods

Subject Selection
The study population consisted of 10 subjects with hypertension (systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg), 12 subjects with hypercholesterolemia (fasting cholesterol >75th percentile for age and gender), 10 active cigarette smokers (average of 1 pack/d for 3 years), and 11 healthy, age-matched controls. Subjects were recruited by advertisement in local newspapers and provided written, informed consent. Subjects were screened by history, physical examination, and routine biochemical analyses. Subjects with risk factors were excluded if they...
had >1 cardiac risk factor, evidence of coronary or peripheral vascular disease, or any other systemic disease. Subjects taking hypertensive medications were asked to discontinue their medications at least 1 week before study initiation. None of the hypercholesterolemic subjects had ever been treated with lipid-lowering medications. None of the healthy subjects had evidence of any cardiac risk factor, cardiovascular disease, or any other systemic illness. The Human Investigational Review Board at Brigham and Women’s Hospital approved the study protocol.

Study Design
All studies were performed in a quiet, temperature-controlled (22°C) room with the subject supine and in the postabsorptive state. Each study consisted of a BQ-123 (Clinalfa AG) infusion into the brachial artery of the nondominant arm. After a minimum of 30 minutes, baseline forearm blood flow (FBF) was measured bilaterally. Once baseline measurements were established, BQ-123 was infused through the intra-arterial catheter at a rate of 100 nmol/min during the infusion. Cyclooxygenase inhibitors and vitamin supplements were prohibited for 1 week before the study. Subjects refrained from alcohol and caffeine for 24 hours before the study. No restrictions were placed on cigarette use before the study.

Vascular Measurements
FBF was measured simultaneously in both arms by venous occlusion, mercury-in-Silastic, strain-gauge plethysmography (D.E. Hoxkinson, Inc).26 Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 40 mm Hg. In addition, a wrist cuff was inflated to suprasystolic pressures 1 minute after the brachial artery of the nondominant arm. After a minimum of 30 minutes, baseline forearm blood flow (FBF) was measured bilaterally. Once baseline measurements were established, BQ-123 was infused through the intra-arterial catheter at a rate of 100 nmol/min for a period of 80 minutes. FBF was measured every 10 minutes during the BQ-123 infusion.

Statistical Analysis
All values are expressed as mean ± SEM. Comparison of baseline characteristics between each risk factor group and healthy controls was made with the Student t test or Fisher exact test. To account for potential systemic effects, the results are expressed as the ratio of FBF in the infused versus the noninfused arm at each time point. Within each group, the effect of BQ-123 on FBF for the 80-minute infusion was assessed by using a generalized least-squares model with random effects that accounted for repeated measurements in individuals. Comparisons of the response to BQ-123 between each risk factor and healthy subjects were made by using group-time interaction terms from separate, generalized least-squares models. Because the changes were linear over time, time was also modeled as a continuous variable. Differences were assessed at the 0.05 level, and all data were analyzed with STATA software (Stata Corp).

Results

Study Population
Baseline characteristics of the study population are presented in the Table. Subjects in each risk factor category were similar to the control population except for the risk factor under study. Baseline FBF was similar in patients in each risk category relative to healthy individuals (Table).

Vascular Responses to ET<sub>A</sub> Receptor Blockade
There were small changes in mean arterial pressure (∆3±1 mm Hg, P=0.001) and heart rate (3±1 bpm, P=0.006) after 80 minutes of BQ-123 infusion, but FBF did not change significantly in the noninfused arm of any subject group (data not shown). FBF increased significantly from baseline in healthy subjects during the 80 minutes of BQ-123 infusion (P=0.007). BQ-123 also caused progressive vasodilation in subjects with hypertension (P<0.001) and hypercholesterolemia (P<0.001). In contrast, BQ-123 infusion did not cause significant forearm vasodilation in cigarette smokers (P=0.185). The average blood flow response in each risk factor group is shown in Figure 1. Evaluation of the FBF response with time as a categorical variable (10-minute increments) revealed that both hypertensive and hypercholesterolemic individuals dilated significantly after 20 minutes of BQ-123 administration, whereas the response in healthy individuals was more variable (Figure 2).

Analysis comparing the flow ratios in each risk factor category to the flow ratios in healthy individuals revealed that only subjects with hypertension vasodilated (46±7%) significantly more in response to BQ-123 than healthy controls (P=0.012). BQ-123 increased flow ratios in both hypercholesterolemic (24±5%) and healthy (20±8%) subjects to a similar extent (P=0.826). Smokers vasodilated the least (10±8%) to BQ-123, and this response was not significantly
Increased Endogenous ET-1 Activity in Hypertension

The observation that selective ET$_A$ receptor blockade causes greater vasodilation in the resistance vessels of hypertensive individuals than in healthy controls is in agreement with previously published data. Both selective ET$_A$ and nonselective ET$_{AB}$ receptor antagonism has been shown to increase forearm vasodilation in hypertensive compared with normotensive subjects.$^{20-22}$ Furthermore, long-term administration of the combined ET$_{AB}$ antagonist bosentan effectively lowers systemic blood pressure in patients with hypertension.$^{27}$ Thus, there is substantial evidence to suggest that ET-1 plays a potential role in the pathophysiology of essential hypertension.

The mechanism by which ET-1 alters vasomotor tone in subjects with essential hypertension remains unclear. Possibly, increased endothelial expression of endogenous ET-1$^{28}$ directly enhances resting tone by activation of vascular smooth muscle cell ET$_A$ and ET$_B$ receptors. In situ hybridization with antisense ET-1 mRNA revealed increased ET-1 mRNA expression in the resistance vessels of subjects with essential hypertension.$^{28}$ In keeping with this finding, some investigators,$^{13,18}$ but not others,$^{29,30}$ have found elevated plasma ET-1 levels in subjects with hypertension. However, the majority of ET-1 is released abuminally, and increased production might not raise plasma levels substantially. The enhanced sensitivity of hypertensive individuals to exogenous ET-1$^{20}$ also raises the possibility that the elevated vascular tone observed in hypertension results from increased ET$_A$ and ET$_B$ receptor expression. There are no studies evaluating ET-1 receptor expression in hypertension, and this question requires further evaluation. Another possibility is a disruption in the balance between the opposing effects of endothelium-derived vasodilatory and vasoconstrictive factors. Thus, if the former were reduced, endothelium-mediated vasoconstriction would be relatively enhanced. This is supported by the observation that vasoconstriction to the NO synthase inhibitor L-N$^G$-monomethyl-arginine (L-NMMA) is significantly decreased in hypertensive patients compared with controls, and the extent of vasodilation in the presence of ET-1 receptor blockade is inversely related to the degree of vasoconstriction with L-NMMA.$^{21}$ Notwithstanding the mechanism, our data provide further evidence that ET-1, acting via ET$_A$ receptors, contributes to increased vasomotor tone in essential hypertension.

Role of Endothelin in Hypercholesterolemia

In the present study, ET$_A$ receptor antagonism with BQ-123 did not cause significant vasodilation in the forearm resistance vessels of hypercholesterolemic subjects relative to healthy volunteers. Animal studies in rats$^{31}$ and pigs$^{32}$ that are fed a high-cholesterol diet have shown that circulating ET-1 levels and ET-1 immunoreactivity are increased in the epicardial coronary arteries and aortas of these animals before the development of atherosclerotic plaques. Oxidized LDLs have also been shown to increase ET-1 mRNA expression in cultured porcine and human aortic endothelial cells.$^{33}$ Similarly, ET-1 levels are elevated in patients with hypercholesterolemia, even in the absence of clinical cardiovascular disease.$^{17}$ Although hypercholesterolemia might increase the endothelial production of ET-1, our results suggest that it might do so below the level of detection measured by changes in FBF.

Figure 1. Average change in FBF after 80 minutes of BQ-123 infusion in the different subject groups. Vascular response is expressed as the ratio of FBF in infused and noninfused arms. FBF increased significantly from baseline in subjects with hypertension and hypercholesterolemia and in healthy volunteers.

Different from that observed in healthy individuals ($P=0.34$). The relative flow ratios in the different risk factor groups are shown in Figure 2.

Discussion

We evaluated the relative contribution of endogenous ET-1 to vasomotor tone in subjects with different cardiovascular risk factors in the absence of clinically evident atherosclerosis. The novel feature and strength of this study is that the contribution of ET-1 to vasomotor function was investigated in subject groups with different atherosclerotic risk factors in a single, laboratory environment that used identical experimental techniques in each group. Our results demonstrate that selective ET$_A$ receptor blockade results in greater vasodilation of the forearm resistance vessels of subjects with hypertension than in hypercholesterolemic subjects or cigarette smokers, relative to healthy controls. These findings suggest that ET-1 plays a greater role in enhancing resting vascular tone in subjects with hypertension compared with other atherogenic risk factors, such as hypercholesterolemia and cigarette use.

In Figure 2, percent change in FBF from baseline over time in each of the subject groups. Vascular response is expressed as the ratio of FBF in infused and noninfused arms. FBF increased significantly in hypertensive subjects relative to healthy controls. FBF did not change significantly in hypercholesterolemic individuals or in cigarette smokers compared with healthy volunteers.

Figure 2. Percent change in FBF from baseline over time in each of the subject groups. Vascular response is expressed as the ratio of FBF in infused and noninfused arms. FBF increased significantly in hypertensive subjects relative to healthy controls. FBF did not change significantly in hypercholesterolemic individuals or in cigarette smokers compared with healthy volunteers.
The degree of vasodilation observed with ET<sub>A</sub> blockade in this study is similar to that reported previously in hypercholesterolemic individuals. However, unlike the study by Cardillo et al., we failed to show a significant difference between hypercholesterolemic and healthy individuals. This discrepancy likely reflects the lack of vasodilation in healthy subjects with BQ-123 in the previous study. Endogenous ET-1 has been implicated in the maintenance of resting vascular tone, and several studies have shown that ET<sub>A</sub> antagonism dilates resistance vessels in healthy individuals. Therefore, our results suggest that in subjects without advanced atherosclerosis, hypercholesterolemia is not associated with increased endogenous ET-1 activity above that observed in the resistance vessels of healthy individuals.

ET-1 might still contribute to heightened vascular tone in the coronary circulation of patients with hypercholesterolemia. Although ET-1 was initially discovered as a product of endothelial cells, it is also secreted by macrophages and activated vascular smooth muscle cells. Recent evidence evaluating the expression of coronary ET-1 at different stages of atherosclerosis reveals that ET-1 expression increases with progression of atherosclerotic lesions, and ET-1 immunoreactivity is particularly elevated in the presence of inflammation. Indeed, we have previously shown that endogenous ET-1 contributes to vasoconstrictor tone in atherosclerotic coronary arteries. Because forearm resistance vessels are relatively protected from atherosclerosis and the accompanying inflammatory process, it is plausible that ET-1 activity is not significantly enhanced in peripheral resistance vessels compared with the conduit coronary circulation.

**ET-1 and Vascular Dysfunction in Smokers**

This, to the best of our knowledge, is the first study that has evaluated the contribution of endogenous ET-1 to vascular tone in the peripheral resistance vessels of chronic cigarette smokers. We found that the vasodilatory response to BQ-123 was statistically similar in smokers and healthy individuals, indicating that ET-1 activity is not upregulated in the forearm resistance vessels of cigarette smokers.

Endothelial dysfunction, manifested as impaired, endothelium-dependent, NO-mediated vasodilation, is associated with both passive and active cigarette smoking. Some studies have found that plasma ET-1 levels are elevated in both light (1 to 10 cigarettes/d) and heavy (10 to 40 cigarettes/d) smokers in a dose-dependent fashion. However, others have shown that smoking is associated with an acute but transient elevation in ET-1 levels and that only acute, but not chronic, exposure to cigarette smoke results in an exaggerated vasoconstrictive response to exogenous ET-1 relative to controls. The lack of significant vasodilation with BQ-123 in this study suggests that, compared with hypertension, ET-1 does not contribute substantially to vascular tone in smokers, especially via the ET<sub>A</sub> receptor. All of the smokers in this study were considered heavy smokers, because they consumed between 10 and 30 cigarettes/d. Thus, it is unlikely that the lack of vasodilation with BQ-123 was attributable to low cigarette use. The present study evaluated the results of chronic smoking and thus, might have missed the vasoconstrictive effects of transient increases in endogenous ET-1 associated with acute cigarette use. It is possible that acute elevations in ET-1 after cigarette consumption may contribute to adverse cardiovascular outcomes by promoting transient vasospasm at preexisting atherosclerotic sites where, in addition to endothelial cells, ET-1–producing inflammatory cells might be more abundant.

**ET-1 and Other Atherogenic Risk Factors**

We did not attempt to survey the contribution of ET-1 in all of the many established and novel atherogenic risk factors. We chose to focus on hypertension, hypercholesterolemia, and cigarette smoking, because available basic science and clinical evidence suggested that ET-1 might play a role in these well-established atherogenic risk factors. The contribution of ET-1 is less clear with other risk factors. For example, children with a family history of premature coronary artery disease do not have elevated ET-1 levels, and hyperhomocysteinemia, though an established risk factor for atherosclerosis, reduces ET-1 expression in cultured human endothelial cells in vitro. Although insulin has been shown to induce ET-1 expression in human endothelial cells in vitro, there are conflicting reports regarding elevated ET-1 levels in diabetic patients. One study evaluating the effect of ET<sub>A</sub> blockade in the forearm resistance vessels of patients with diabetes mellitus did not show any vasodilation with BQ-123 infusion, whereas another did suggest increased endogenous ET-1 activity in type 2 diabetics. Still other studies suggest that elevations in plasma ET-1 levels are directly correlated with the presence of microvascular complications, and it is possible that in diabetes, like hypercholesterolemia and cigarette smoking, ET-1 activity is upregulated only in the presence of local inflammation.

**Limitations**

At the dose used in this study, BQ-123 caused a small but significant change in heart rate and mean arterial pressure. However, BQ-123 did not significantly alter FBF in the noninfused forearm. To account for the systemic effects of BQ-123, we analyzed the data as a ratio of flows in the infused and noninfused forearms, rather than as flows in the infused forearm alone. Another limitation of this study is the small number of patients in each risk factor category. We cannot exclude the possibility that we might have detected a significant vasodilator response in smokers had we enrolled more patients. However, the purpose of this study was to examine the relative importance of ET-1 in a range of risk factors. A power calculation based on the results of this study showed that we would need to enroll an additional 430 subjects per group and an additional 626 subjects per group, respectively, to show a significant difference in the response between hypercholesterolemic subjects and cigarette smokers relative to healthy volunteers. Despite the small numbers, we have clearly shown the greater importance of ET-1 in the pathophysiology of hypertension. An additional limitation is that we excluded any subjects with clinical evidence of atherosclerosis. It is possible that in the presence of advanced atherosclerosis, ET-1 bioavailability, predominantly from macrophages, is enhanced and vasoconstriction ensues, regardless of which risk factor is present. Therefore, the
varying effects of ET-1 blockade might not hold true in patients with advanced atherosclerosis and different atherogenic risk factors.

Conclusions

In conclusion, ET-1 plays a significant role in the pathophysiology of hypertension compared with that of other major cardiovascular risk factors in subjects without clinically overt atherosclerosis. ET-1 blockade might be particularly beneficial in patients with early and late manifestations of hypertension by interfering with an endogenous mediator of increased vasomotor tone. However, any sustained benefits of ET-1 antagonism in patients with hypertension must be evaluated in long-term safety and efficacy trials.

Perspectives

Our findings show that the contribution of ET-1 to vasomotor dysfunction might vary in patients with different atherogenic risk factors. ET-1 is particularly relevant to increased vascular tone in patients with hypertension, and as such, might be an important pathogenic mechanism for the development of high blood pressure. In patients with hypertension, ET-1 compounds other abnormalities in endothelial function, such as the decreased bioavailability of NO. In contrast, ET-1 plays a less important role in the peripheral resistance vessels of subjects with other risk factors, such as hypercholesterolemia and in cigarette smoking. Yet in the presence of atherosclerosis, ET-1 might be an important mediator for vascular dysfunction, irrespective of the risk factor profile.

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