Systemic Blockade of the Endothelin-B Receptor Increases Peripheral Vascular Resistance in Healthy Men

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Abstract—Endothelin-1 (ET-1) is an important mediator of vascular tone in humans, and a number of endothelin receptor antagonists are currently in clinical development as vasodilator agents. While the vasoconstrictor role of the ET\textsubscript{A} receptor is undisputed, the role of the ET\textsubscript{B} receptor remains unclear. Hemodynamic effects of systemic doses of the ET\textsubscript{B}-selective antagonist BQ-788 were investigated in 5 healthy male volunteers (age range, 33 to 48 years) in a placebo-controlled, four-way crossover study. After a 15-minute infusion of BQ-788 (3, 30, or 300 nmol/min) or placebo, plasma ET-1 and big ET-1, blood pressure, heart rate, cardiac index, and stroke index were measured. Total peripheral vascular resistance was calculated from cardiac index and mean arterial pressure. Hemodynamic data are expressed as maximum, placebo-corrected, percentage change from baseline following BQ-788 (300 nmol/min) and were examined by ANOVA. Plasma ET-1 increased by 3.7±1.2 pg/mL (maximum at 15 minutes, \(P=0.02\)), whereas there was no significant change in plasma big ET-1. Although BQ-788 had no effect on mean arterial pressure, there was a reduction in heart rate (13±7% at 50 minutes; \(P=0.002\)), cardiac index (17±5% at 40 minutes; \(P<0.0001\)), and stroke index (8±4% at 40 minutes; \(P=0.002\)) and an increase in total peripheral vascular resistance (24±5% at 40 minutes; \(P<0.0001\)). The selective ET\textsubscript{B} receptor antagonist BQ-788 causes peripheral vasconstriction in healthy volunteers, suggesting that the overall balance of effects of endogenous ET-1 at the vascular ET\textsubscript{B} receptor favors vasodilatation. Further investigation is now clearly required to address whether selective ET\textsubscript{A} or combined ET\textsubscript{A}/ET\textsubscript{B} receptor blockade will be more effective in the clinical setting. (Hypertension. 1999;33[part II]:581-585.)

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endothelin receptor antagonist

The importance of endothelin-1 (ET-1) as a mediator of basal vascular tone in vivo in humans has been demonstrated by local and systemic vasodilatation in response to endothelin receptor antagonism. The potent vasoconstrictor effects of ET-1 combined with the increased plasma concentrations of ET-1 associated with cardiovascular diseases, including heart failure and renal failure, provide strong evidence to support a functional role for ET-1 in the development and maintenance of the increased peripheral vascular resistance associated with these conditions.

The vascular effects of ET-1 are mediated by two distinct receptors: the ET\textsubscript{1}–selective ET\textsubscript{A} receptor and the nonisopeptide-selective ET\textsubscript{B} receptor. The sustained vasoconstrictor effects of ET-1 are predominantly mediated by the ET\textsubscript{A} receptor, although vascular smooth muscle ET\textsubscript{B} receptors have also been described and may, under some circumstances, contribute to ET\textsubscript{1}–mediated vasoconstriction in animal models and humans in vivo. ET\textsubscript{B} receptors were first described on endothelial cells, where they act to modulate the vasoconstrictor effects of ET-1 through generation of nitric oxide and prostacyclin. The ET\textsubscript{B} receptor also has a role in the clearance of ET-1 from the circulation, although the exact site of the clearance receptor remains to be confirmed. The contribution of the vascular ET\textsubscript{B} receptor to the recognized endogenous ET-1–mediated constrictor tone depends on the balance between the ET\textsubscript{B} receptor–mediated effects of vasodilatation, vasoconstriction, and ET-1 clearance.

Local vasoconstriction to ET\textsubscript{B} receptor agonists has been described in healthy volunteers and in patients with heart failure. However, more recently, vasoconstriction after local administration of the selective ET\textsubscript{B} receptor antagonist BQ-788 has been described in healthy volunteers and in patients with heart failure. The results with antagonists are particularly important as they indicate that the endogenous effect of vascular ET\textsubscript{B} receptor stimulation in vivo favors vasodilatation. Indeed, hypertension has been described after administration of systemic doses of the selective ET\textsubscript{B} receptor antagonists A192621 in rats and BQ-788 in rabbits in vivo, as well as in rescued ET\textsubscript{B} knockout mice. The vasoconstrictor effects of ET\textsubscript{B} antagonists may result directly from blockade of an endothelial ET\textsubscript{B} receptor–mediated dilator...
tone or indirectly from displacement of endogenously generated ET-1 to vasoconstrictor ET\(_A\) receptors, or as a result of reduced clearance of ET-1 by vascular ET\(_B\) receptors. Confirmation of the balance of the vascular effects mediated by the ET\(_B\) receptor in different circumstances is important in understanding the physiology of the endothelin system and in determining whether selective ET\(_A\) receptor antagonists or combined ET\(_A/ET_B\) receptor antagonists are likely to be more effective vasodilator agents in the clinical setting. Although both selective and nonselective endothelin receptor antagonists have demonstrated vasodilator effects in healthy subjects,\(^1,2\) in patients with heart failure,\(^22,23\) and in patients with hypertension,\(^24,25\) the question of whether selective ET\(_A\) or combined ET\(_A/ET_B\) receptor antagonism will be of more benefit as vasodilator therapy remains to be clarified.

As a first step in understanding the contribution of the ET\(_B\) receptor to the maintenance of vascular tone in vivo, we investigated the systemic hemodynamic effects of BQ-788 in healthy male volunteers.

**Methods**

**Subjects**

Five healthy male subjects between 18 and 50 years of age were recruited to the study, which was performed in the Clinical Research Center at the Western General Hospital, Edinburgh, with the approval of the local research ethics committee and the written informed consent of each subject. The investigations conformed with the principles outlined in the Declaration of Helsinki. No subject received vasoactive medication or nonsteroidal anti-inflammatory drugs in the week before each phase of a study, and all subjects abstained from alcohol for 24 hours and from food, caffeine-containing drinks, and tobacco for at least 4 hours before any measurements were made. All studies were performed in a quiet room kept at a controlled temperature between 22°C to 24°C.

**Drugs**

BQ-788 (Clinalfa AG) was used as a selective ET\(_B\) receptor antagonist on the basis of both a 1000-fold selectivity of BQ-788 for the ET\(_B\) receptor, in the nanomolar range, in human cell lines\(^18\) and inhibition of ET-3 binding to recombinant human ET\(_B\) receptors expressed in Chinese hamster ovary cells, also in the nanomolar range.\(^26\) The dose range (3 to 300 nmol/min) used in the current study was selected from previous work investigating the local effects of BQ-788 in the forearm circulation\(^3\) and from a dose ranging pilot study in which 2 volunteers were studied at each dose level (data not shown). Selected doses (1 to 300 nmol/min) were administered in the pilot study to identify a no-effect dose and select an appropriate maximum dose for the main study.

BQ-788 was dissolved in physiological saline (0.9%, Baxter Healthcare, Ltd). Saline (0.9%, Baxter Healthcare, Ltd) was administered as placebo. BQ-788 and placebo were administered in a single-blind manner and infused intravenously at a constant rate for 15 minutes via an 18 standard wire gauge (SWG) cannula sited in the left antecubital vein. All solutions were prepared from sterile stock solutions on the day of the study.

**Measurements**

**Plasma ET-1 and Big ET-1**

Blood samples were obtained before dose and at 5, 15, 60, and 240 minutes after dose via an 18 SWG cannula sited in the noninfused arm. In brief, 10-mL samples were collected into sterile EDTA tubes (K3 EDTA, Vacutainer, Becton Dickinson Vacutainer Systems), centrifuged immediately at 2000 rpm for 20 minutes, and stored in plain tubes at −80°C before assay. ET-1 and big ET-1 (Peninsula Laboratories Europe) were determined by standard radioimmunoassay, as previously described.\(^27,28\)

Blood samples were also taken on admission and before discharge for routine biochemistry and hematology blood tests (sodium, potassium, creatinine, urea, alkaline phosphatase, γ-glutamyl transpeptidase, hemoglobin, and white cell count).

**Hemodynamic Recordings**

Hemodynamic recordings were made at 10-minute intervals from 30 minutes before dose until 1 hour after the start of the infusion, with an additional blood pressure measurement at 15 minutes corresponding with the end of the infusion. Recordings were again made at 30-minute intervals until 2 hours and hourly until 4 hours after the start of the infusion.

Blood pressure and heart rate (HR) were recorded in duplicate at each time point using a semi-automated noninvasive oscillometric method in the noninfused arm (Takeda UA 751 sphygmomanometer, Takeda Medical Inc)\(^29\); values were averaged for each time point. Blood pressure is presented as mean arterial pressure (MAP; diastolic blood pressure + 1/3 pulse pressure, in millimeters of mercury).

Cardiac output and stroke volume were recorded by a well-validated noninvasive bioimpedance technique (NCCOM3; BoMed Medical Manufacturer Ltd).\(^30\) These parameters were corrected for body surface area and described as cardiac index (CI, liters per minute per meters squared) and stroke index (SI, milliliters per meter squared). Total peripheral vascular resistance index (TPVRI) was calculated as MAP divided by CI and expressed in arbitrary units (AU).

**Study Design**

Responses to BQ-788 (3, 30, and 300 nmol/min) and placebo were investigated in a placebo-controlled, four-way crossover study. Study drugs were administered in a single-blind manner. The order of treatments was randomized. Five subjects attended for 4 separate study visits, each separated by at least 5 days. Subjects were resident in the research center for at least 6 hours. Subjects rested supine for at least 20 minutes before hemodynamic measures, and baseline measures were made in the 30 minutes before study drug administration.

**Analysis**

Plasma ET-1 and big ET-1 are represented as absolute change from predose (picograms per milliliter), with statistical significance assessed by paired \(t\) test. Hemodynamic results are expressed as maximum placebo-corrected percentage changes from baseline ±SEM.\(^2\) Statistical analysis was performed on untransformed data. Responses were examined by repeated-measures ANOVA. Statistical significance was taken at the 5% level, and analysis was performed using an Excel data analysis package (Excel 5.0, Microsoft Ltd).

**Results**

All 5 healthy male subjects (age range, 33 to 48 years) completed the study. No adverse events were reported, and there were no clinically relevant changes in routine biochemistry and hematology blood tests.

**Plasma ET-1 and Big ET-1**

Predose plasma ET-1 concentrations did not differ significantly for any of the treatments (range of baseline mean values, 4.4 to 4.9 pg/mL). Plasma ET-1 concentration increased significantly after administration of BQ-788 (from 4.6±0.8 to 8.4±1.8 pg/mL at 15 minutes with 300 nmol/min, \(P<0.02\)) but not during treatment with the lower doses of BQ-788 or placebo (Figure 1). In contrast, concentrations of big ET-1 did not change significantly with treatment.
Baseline measurements for hemodynamic parameters during the placebo treatment period were as follows: MAP, 79±3 mm Hg; HR, 79±3 bpm; CI, 2.6±0.2 (L/min/m²); SI, 49±3 mL/m²; and TPVRI, 31.1±1.8 AU. Baseline values were similar for each of the other treatment periods. MAP did not alter significantly after administration of BQ-788 at any dose (3±2% at 90 minutes with 300 nmol/min; P=0.4) (Figure 2). After administration of BQ-788, there were changes in all other hemodynamic parameters when compared with placebo that appeared to be dose-related and that were significant at 300 nmol/min; HR decreased (13±7% at 50 minutes after dose; P=0.002), CI decreased (17±5% at 40 minutes; P<0.0001), and there was a small reduction in SI (8±4% at 40 minutes; P=0.002). TPVRI increased (24±5% at 40 minutes; P<0.0001).

Discussion

We have demonstrated substantial systemic vasoconstriction, associated with a reduction in HR and CI but no change in MAP, in response to administration of the selective ETB receptor antagonist BQ-788 in healthy men. Consistent with our earlier work in the forearm circulation, these observations are highly suggestive of the overall effect of endogenous ETB receptor–mediated vascular tone favoring vasodilatation. An alternative explanation for the hemodynamic effects is that BQ-788 is directly negatively chronotropic and that peripheral effects are indirect. However, this is unlikely given our earlier work and the lack of evidence of an important positive chronotropic and inotropic role of the cardiac ETB receptor. Although peripheral resistance was substantially increased, blood pressure was unaffected because of a decrease in HR that was probably reflex in origin. We have also demonstrated increases in plasma ET-1, but not big ET-1, concentrations after ETB receptor blockade, consistent with reduced clearance of ET-1 by the ETB receptor. All of these effects were prominent with BQ-788 at the highest dose but were not clearly seen at lower doses.

The vasoconstrictor effects of ETB receptor antagonism may result directly from blockade of the vasodilator effects of the endothelial ETB receptor or indirectly from displacement of endogenously generated ET-1 from ETB receptors to unoccupied ETA receptors. It is unlikely that these effects are mediated by nonselective ETa/ETB receptor blockade because they are the opposite of those found with selective ETA receptor antagonists in healthy subjects (unpublished data, 1998) and patients with heart failure and of those found with combined ETa/ETB receptor antagonists in healthy subjects. Clearly, the indirect effects of ET-1 on ETA receptors are more relevant with administration of selective ETB antagonists than with nonselective ETa/ETB receptor antagonists, because in this latter situation the constrictor ETA receptor is also blocked. Indeed, vasodilator effects have been demonstrated with both selective and nonselective antagonists.
endothelin receptor antagonists in humans, and the nonsel-
selective ET₄/ET₆ antagonist bosentan has recently been shown to
effectively lower blood pressure in patients with hyperten-
sion.²⁵ However, direct comparison of the effects of selective
and nonselselective endothelin receptor antagonism will be
important in assessing the relative contribution of each
receptor subtype to the vascular effects of ET-1.

We and others have previously demonstrated forearm
vasodilatation in response to local ET₆ receptor antagonist
with BQ-123.¹³,²²,²³ In the presence of BQ-788 in healthy
volunteers, this effect was attenuated,³ suggesting that the
overall effect of vascular ET₆ receptor stimulation by endo-
genous ET-1 is vasodilatation. This attenuation of BQ-123–
mediated vasodilatation by BQ-788 suggests that the vaso-
constrictor effect of ET₆ receptor blockade is not mediated by
displacement of ET-1 onto the ET₆ receptor but is due to
direct blockade of ET₆-mediated vasodilator tone. We have
also shown, using a “nitric oxide clamp” technique, that the
vasodilator response to BQ-123 is in part mediated by nitric
oxide³ and, therefore, probably mediated by the endothelial
ET₆ receptor. Loss of endothelial cell ET₆–mediated vasodi-
lator tone may occur in cardiovascular diseases, such as
essential hypertension and hypercholesterolemia, in which is
associated endothelial dysfunction.³³,³⁴ Here, because of a
reduced capacity for ET₆ receptor–mediated, nitric
oxide–dependent dilatation, selective ET₆ receptor antago-
nists may be less effective.

In summary, we have demonstrated systemic vascon-
striction in response to acute ET₆ receptor blockade with the
selective ET₆ receptor antagonist BQ-788 in healthy
men in vivo, indicating that the predominant endogenous
effect of stimulating vascular ET₆ receptors is vasodilata-
tion. One exciting possibility is that tonic endogenous
ET-1 release, acting via the endothelial ET₆ receptor, is
responsible for the physiological basal release of nitric
oxide. This now needs to be addressed in clinical studies.
Further investigation of the influence of ET₆ receptor
antagonism on the sympathetic nervous system and renal
function are also warranted. In addition, direct comparison
of the effects of chronic administration of selective ET₆
and combined ET₄/ET₆ receptor antagonists is required in
patients with cardiovascular disease, with and without
endothelial dysfunction, in order to confirm which of these
approaches is likely to be more effective in the clinical
setting.

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