Action of the endothelin receptor (ET\textsubscript{A}) antagonist BQ-123 on forearm blood flow in young normotensive subjects

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ABSTRACT

Endothelin-1 (ET-1) has been proposed to contribute to the regulation of vascular tone in humans. BQ-123, an ET\textsubscript{A} receptor antagonist, has also been reported to increase forearm blood flow (FBF) \textit{in vivo}; however, the efficacy of BQ-123 as an antagonist of ET-1 has not been evaluated in the forearm. The present study investigated the effects of BQ-123 on changes in FBF in response to ET-1 and noradrenaline (NA; norepinephrine), taking into account the possible influence of vasodilator effects of BQ-123 on responses to vasoconstrictors. Six subjects (age 25–34 years) participated in a double-blind randomized study. FBF was measured by forearm occlusion plethysmography. Drugs were infused intra-arterially into the non-dominant arm (study arm) on four separate occasions; the non-infused arm was used as a control. The effects of BQ-123 (50 nmol/min for 60 min, or 300 nmol/min for 5 min followed by saline for 55 min) were compared with the effects of infusion of sodium nitroprusside (SNP; 12 nmol/min for 60 min) or saline on vasoconstriction induced by ET-1 (10 pmol/min for 7 min) and NA (120 pmol/min for 7 min). Infusion of BQ-123 at either dose did not significantly increase FBF, whereas SNP increased FBF by 134\% (P = 0.03). ET-1 significantly reduced FBF, and this effect was almost completely inhibited by both doses of BQ-123, but was unaffected by SNP. NA also reduced FBF, and this action was unaffected by BQ-123 or SNP. The data show that BQ-123 is a selective ET-1 antagonist, but do not confirm a major role for ET-1 in influencing resting forearm vascular tone in young normotensive subjects.

INTRODUCTION

Endothelin-1 (ET-1) is a 21-amino-acid peptide produced by endothelial cells that has potent vasoconstrictor actions [1]. Under normal circumstances, circulating levels of ET-1 are low (< 2 pg/ml) [2], but elevated levels have been reported in pre-eclampsia [3], pulmonary hypertension, arteriosclerosis, renal failure, acute coronary syndromes, heart failure, migraine and other vascular diseases [4], implying a possible role for ET-1 in these conditions. It has been suggested that, despite its low circulating levels, ET-1 normally plays a paracrine role in the regulation of vascular tone in humans. This is supported by the observations that non-selective endothelin (ET\textsubscript{A} and ET\textsubscript{B}) receptor antagonists reduce systemic blood pressure [5–8], and that the selective endothelin ET\textsubscript{A} receptor antagonist BQ-123 increases forearm blood flow (FBF) \textit{in vivo} [8–11].

Key words: blood flow, BQ-123, endothelin, noradrenaline, plethysmography, sodium nitroprusside, vasodilation.
Abbreviations: ET-1, endothelin-1; FBF, forearm blood flow; NA, noradrenaline; SNP, sodium nitroprusside.
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Interestingly, none of these forearm studies have confirmed the antagonist action of BQ-123 against ET-1 at the doses used. Haynes and Webb [10] did show that the vasodilator effect of BQ-123 could be attenuated by co-infusion of ET-1; however, since BQ-123 alone increased FBF, it was not clear whether this finding reflected pharmacological antagonism or functional antagonism resulting from the summation of the simultaneous vasodilator and vasoconstrictor effects of the two agents. Moreover, the possibility that the increased blood flow secondary to BQ-123 action might influence responses to ET-1 [12] was not explored.

Consequently, the present study was undertaken to investigate the ability of BQ-123 to antagonize ET-1-induced vasoconstriction in the forearm, while controlling for possible confounding effects of BQ-123-induced increases in FBF on responses to ET-1.

METHODS

Subjects

The study was approved by the local ethics committee, and adhered to the ‘Declaration of Helsinki’ principles of clinical research. Written, informed consent was given by each subject. The study design was double-blind and randomized. A total of 24 drug infusion protocols were performed in six healthy volunteers (age 25–34 years). Subjects were asked to abstain from alcohol for 24 h and from caffeine or cigarettes for 3 h prior to each infusion protocol.

Forearm plethysmography

Studies were performed in a quiet room, maintained at a constant temperature of 25 °C. A 23G Wallace Y-Can cannula was inserted into the brachial artery of the non-dominant arm under local anaesthetic (lignocaine 1%), and physiological saline or drug was infused at a rate of 0.5 ml/min. The side arm of the cannula was connected to a pressure transducer (SenorNor 840; AD Instruments, Castle Hill, NSW, Australia) via a pressure dome (SensorNorasa; Medanco Products, Munster, Germany) to measure intra-arterial pressure continuously. The subjects lay in a supine position, with both arms supported in a pronated position on foam cushions just above the level of the right atrium, with inflatable cuffs placed around each wrist and upper arm. FBF (ml min⁻¹ 100 ml⁻¹ forearm) was measured using venous occlusion plethysmography, as described by Whitney [13]. A mercury-filled Silastic strain gauge was placed around the widest part of each forearm and the gauge was connected to a plethysmograph (EC-6; D. E. Hokanson, Bellevue, WA, U.S.A.). Once the subject was comfortable, the gauge was calibrated to measure the percentage change in the volume of the forearm following occlusion of forearm venous drainage. The rate of change of forearm volume following venous occlusion is directly proportional to arterial inflow. At 2 min before each measurement, the wrist cuffs were inflated to 200 mmHg with a rapid cuff inflator (EC20; D. E. Hokanson) to exclude the circulation in the hands. FBF measurements were made four times per min by rapidly inflating the upper-arm cuffs to 40 mmHg for 10 s intervals, with 5 s of subsequent deflation, for a total of 3 min. The mean of the last four measurements was used for analysis.

Study protocols

The study was designed in two sets, each performed in a balanced random order on occasions separated by at least 1 week (Figure 1). Randomization was achieved using randomization tables. The first set of studies investigated the effects of BQ-123 (Clinalfa, Calbiochem-Novabiochem Ltd, Nottingham, U.K.) and of the co-infusion of noradrenaline (NA; norepinephrine) (Abbott Laboratories) or ET-1 (Clinalfa) and BQ-123 on FBF. Once the brachial cannula had been inserted, saline was infused at 0.5 ml/min for 30 min. FBF was measured in both the control and study arms for the last 3 min of each infusion period. In one study protocol the saline was then replaced with an infusion of BQ-123 at 50 nmol/min for 60 min, with 3 min measurements of FBF every 10 min. This was followed by a co-infusion of NA (120 pmol/min) and BQ-123 for 7 min, with a subsequent washout period of BQ-123 (50 pmol/min) alone for 10 min, and finally a co-infusion of BQ-123 (50 pmol/min) and ET-1 (10 pmol/min) for 7 min. The dose of BQ-123 was chosen on the basis that Verhaar et al. [11] and Berrazaeta et al. [9] have demonstrated that BQ-123 infused at 10 nmol/min for 60 min or 50 nmol/min for 5 min (followed by saline) respectively induced a maximum vasodilatation of 60% after 60 min, which is similar to that induced when BQ-123 is infused at 100 nmol/min for 60 min [10,14]. Therefore it was anticipated that BQ-123 infused at 50 nmol/min for 60 min would induce a similar magnitude of vasodilatation. The doses of NA and ET-1 were chosen because they have been reported to induce comparable reductions in FBF [15].

On the basis of previous reports [6,9,10,14], it was anticipated that BQ-123 would increase FBF by approx. 60%. Therefore, in the other limb of the protocol, in order to control for any effects on responses to ET-1 or NA resulting from the increased FBF, sodium nitroprusside (SNP; David Bull Laboratories, Warwick, U.K.) was infused at 12 nmol/min, and its effects on FBF responses to NA and ET-1 were measured. This dose was anticipated to cause an approx. 60% increase in FBF on the basis of published studies [16].

The second set of study protocols investigated the effects of a higher dose of BQ-123 on FBF and on
Effect of BQ-123 on forearm blood flow

Figure 1  Diagramatic representation of the study protocol
Intra-arterial drugs were infused for the periods indicated by blocks: BQ-123 at 50 nmol/min (BQ-12350) and 300 nmol/min (BQ-123300), SNP at 12 nmol/min, NA (NE) at 120 pmol/min and ET-1 at 10 pmol/min.

the responses to NA and ET-1 (Figure 1). In one limb of this study protocol, after the initial saline infusion, BQ-123 at a dose of 300 nmol/min was infused for 5 min, followed by saline for 55 min, while measuring FBF every 10 min. This was followed by infusions of NA (120 pmol/min) and ET-1 (10 pmol/min) separated by a 10 min saline washout. FBF was measured for the last 3 min of each infusion. In the second limb of this study set, the effects of NA and ET-1 on FBF were measured after 60 min of saline alone.

Analysis and statistics
The sample size calculations were based on the assumption that the study should be powered to detect a ≥ 15% change in FBF. Both Cardillo et al. [14] and Haynes and Webb [10] reported a 20% change in vascular flow after ET-1 was infused at 5 pmol/min for 10 min. Previous control studies indicated that the expected S.D. of FBF would be ~7%. Therefore a study sample of six subjects was required in order to achieve a power of ≥ 85% at the 95% significance level. Power calculations were performed using Gpower 2.0 [16a].

Changes in FBF in the study arm were adjusted with respect to changes in the control arm [17]. The percentage change in FBF in the study arm was calculated by measuring the change in the ratio of flow in the study arm (FBF_S) to that in the control arm (FBF_C) before and after infusion of the drug (Δ[FBF_S/FBF_C]), and taking this as a percentage of the ratio of flow in the study arm to that in the control arm prior to infusion of the drug. These results are shown as means (95% confidence intervals). Statistical analysis was performed using Instat 3.3 (GraphPad Inc.), and significance testing was performed using Friedman’s non-parametric one-way ANOVA for repeated measures followed by the least significant difference post hoc test; P < 0.05 was taken to indicate statistical significance.

RESULTS
There were no significant changes in mean systolic or diastolic blood pressure, mean heart rate or FBF in the non-infused arm after infusion of any of the drugs used in the different studies. There were no differences in the mean basal blood flow or resistance between the control arm and study arm (Table 1).

Compared with saline infusion [mean increase in FBF of 1% (95% confidence interval −6 to 9%)], BQ-123 at a dose of 50 nmol/min for 60 min had no significant effect on FBF [increase of 9% (8 to 10%); P = 0.1]. Similarly, infusion of BQ-123 at 300 nmol/min for 5 min did not significantly affect FBF [increase of 1% (−6 to 8%)], and FBF remained unchanged over a subsequent period of 55 min during which saline was infused (results not shown). In contrast, SNP induced a marked increase in FBF [134% (84 to 184%); P = 0.03] (Figure 2).

Infusion of ET-1 caused a reduction in FBF that was not affected significantly by concurrent infusion of SNP [saline, −16% (−21 to −11%); SNP, −13% (−16 to −9%); P = 0.5] (Figure 3). Both dose regimes of BQ-123 almost completely prevented the response to ET-1 [50 nmol/min BQ-123, 0% (−4 to 6%) (P = 0.03); 300 nmol/min BQ-123, 1% (−2 to 1%) (P = 0.03)].
Table 1  Haemodynamic parameters during the studies

The infused agents were: saline, BQ-123 at 50 nmol/min for 60 min (BQ-123_{50}) and at 300 nmol/min for 5 min (BQ-123_{300}), and SNP at 12 nmol/min for 60 min. All data are means (95% confidence intervals); *P < 0.05 compared with saline (Friedman’s non-parametric ANOVA least significant difference test).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline</th>
<th>BQ-123_{50}</th>
<th>BQ-123_{300}</th>
<th>SNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>87 (84–90)</td>
<td>88 (80–95)</td>
<td>100 (91–109)</td>
<td>101 (91–111)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>57 (53–61)</td>
<td>60 (53–67)</td>
<td>62 (54–70)</td>
<td>63 (61–65)</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>67 (64–70)</td>
<td>68 (62–74)</td>
<td>75 (67–83)</td>
<td>76 (72–80)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>72 (68–74)</td>
<td>74 (70–78)</td>
<td>75 (70–80)</td>
<td>73 (70–76)</td>
</tr>
<tr>
<td>FBF in control arm after 60 min (ml min^{-1} 100 ml^{-1})</td>
<td>3.0 (2.3–3.6)</td>
<td>3.1 (2.3–3.8)</td>
<td>2.4 (1.7–3.2)</td>
<td>2.4 (1.0–3.8)</td>
</tr>
<tr>
<td>FBF in study arm after 60 min (ml min^{-1} 100 ml^{-1})</td>
<td>3.1 (2.8–3.5)</td>
<td>3.5 (3.0–4.1)</td>
<td>3.0 (2.8–3.9)</td>
<td>7.1 (5.5–8.7)</td>
</tr>
<tr>
<td>Resistance in control arm after 60 min (dyn s cm^{-5})</td>
<td>25 (22–29)</td>
<td>24 (19–29)</td>
<td>34 (25–42)</td>
<td>37 (18–56)</td>
</tr>
<tr>
<td>Resistance in study arm after 60 min (dyn s cm^{-5})</td>
<td>23 (21–29)</td>
<td>21 (17–25)</td>
<td>26 (20–31)</td>
<td>10 (6–14)</td>
</tr>
</tbody>
</table>

DISCUSSION

The present study confirms that intra-arterial infusion of ET-1 causes forearm vasoconstriction. Although ET-1 is known to induce a slow-onset vasoconstriction, which reaches a maximum approx. 30–45 min after commencement of the infusion, the decrease in FBF seen in the present study was comparable with that reported after 10 min of ET-1 infusion at 5 pmol/min [10,17]. Pre-infusion of BQ-123 at two different dose regimes (50 nmol/min for 60 min or 300 nmol/min for 5 min) antagonized ET-1-induced vasoconstriction effectively, and did not affect the decrease in FBF induced by NA.

Interestingly, there was no significant increase in basal FBF following infusion of BQ-123 alone at either a low or a high dose in the present study, and the upper 95% confidence limit of change with either dose of BQ-123 did not exceed 10%. In view of our findings showing inhibition of the response to infused ET-1, this lack of effect of BQ-123 on resting FBF cannot be attributed to a failure of the drug to act as an ET-1 antagonist. These observations therefore suggest that endogenous ET-1 makes little or no contribution to the control of resting FBF in these young normotensive subjects.

In contrast, some previous studies have suggested that ET-1 plays a role in the maintenance of basal forearm vascular tone, on the basis that infusion of BQ-123 via the brachial artery induced a modest increase in FBF in healthy volunteers [8–11]. However, our findings are similar to those in a study by Cardillo et al. [14], who demonstrated that infusion of BQ-123 at 100 nmol/min for 60 min did not increase FBF in healthy volunteers, although this dose did increase blood flow modestly in hypertensive patients. Similarly, infusion of the ET_{A} specific competitive inhibitor FR 139317 into cat gastrocnemius muscle abolished the constrictor response to 50 nmol/min [−7% (−19 to 6%)]) or BQ-123 at 300 nmol/min [−9% (−16 to −3%); P = 0.57].
ET-1, but had no effect on resting muscle blood flow [18].

The reason for the observed difference in the response to BQ-123 in these studies is unknown, although it could reflect methodological differences. The age range of the volunteers in our study was not markedly different from that in other studies [9–11,14], and all subjects were healthy and normotensive, were not on any other medication and had avoided any vasoactive substance (e.g. food, alcohol, coffee and smoking) for at least 3 h prior to the study. The mean arterial pressure in our study was 72 mmHg (95% confidence interval 71–73 mmHg), which was somewhat lower than that reported by Cardillo et al. [14] (81 ± 2 mmHg; mean ± S.E.M.), and all our volunteers were Caucasian, whereas Cardillo et al. [14] included 13 Caucasian and five Afro-Caribbean volunteers. Data on mean blood pressure or ethnicity were not available from the other studies. It is possible that other variables, such as salt intake or lipid profiles, could be important, but these were not measured in this or the other studies.

Differences in responses to systemic infusions of BQ-123 have also been reported. Schmetterer et al. [19] infused 25 nmol/min for 60 min into the systemic circulation in healthy volunteers and found that there were no renal or systemic haemodynamic effects. In contrast, infusion of BQ-123 at 200 nmol/min through a central venous catheter in patients with chronic heart failure has been reported to reduce mean arterial pressure and systemic vascular resistance, but did not affect pulmonary vascular resistance significantly [20]. This latter observation contrasts with the findings of Prendergast et al. [21], who demonstrated that BQ-123 did decrease pulmonary resistance in infants with pulmonary hypertension following corrective surgery for congenital heart disease. It is possible that some of these discrepancies relate to pathology or a failure to antagonize ETβ receptors, since blockade of both ETα and ETβ receptors with ET-1 antagonists such as bosentan and TAK-044 has been shown to consistently reduce blood pressure and peripheral vascular resistance in both healthy volunteers and patients with cardiovascular disease [5–8].

Variations in responses to endothelin receptor antagonists are also seen in animal studies, with marked differences in responses between as well as within species. Warner et al. [22] demonstrated that BQ-123 infused into anaesthetized normotensive Wistar rats decreased their mean arterial pressure, whereas Douglas et al. [23] showed that BQ-123 had little effect in normotensive Wistar Kyoto rats, but reduced blood pressure in spontaneously hypertensive rats. In contrast, McMahon et al. [24] demonstrated that BQ-123 only lowered arterial pressure in renal hypertensive rats at very high doses.

Similar inconsistencies have also been reported with non-selective ET antagonists. SB 209670, a mixed ETα/ETβ receptor antagonist, had no effect on basal haemodynamic parameters in conscious and anaesthetized Sprague–Dawley rats in one study [25], although prolonged infusion for over 20 h has been reported to reduce mean arterial pressure in the same strain [26]. In contrast, Bunting and Widdop [27] recorded a significant increase in mesenteric and hindquarters blood flow in both spontaneously hypertensive rats and Wistar Kyoto rats in response to infusion of SB 209670.

It is clear that there are variations in the response to BQ-123 in both human and animal studies. These may be due to variations in the expression of receptor subtypes. Love et al. [28] demonstrated that ETβ receptor stimulation using sarafotoxin S6c induced vasoconstriction of the forearm in both healthy volunteers and patients with chronic heart failure. The possibility that variations in the ETα/ETβ ratio in the different volunteer groups account for the differences in the reported responses to BQ-123 may be worthy of further study.

In conclusion, the present study indicates that, at the doses used, BQ-123 acts as a selective ET-1 antagonist in the forearm. Moreover, this effect is maintained for up to 80 min after the infusion of BQ-123. Contrary to some previous studies, BQ-123 did not have a significant effect on resting FBF, and hence we cannot confirm previous observations suggesting that ET-1 contributes to resting forearm vascular tone in young normotensive subjects.

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