Dose- and time-dependency of the dilator effects of the endothelin antagonist, BQ-123, in the human forearm

José R. Berrazueta,¹ Kiran Bhagat,² Patrick Vallance² & Raymond J. MacAllister²

¹Department of Medicine, Cantabria University of Spain, Santander, Spain, ²Centre for Clinical Pharmacology, Cruciform Project, University College London, The Rayne Institute, 5 University Street, London WC1E 6JJ

Aims Endothelin-1 is a potent endogenous vasoconstrictor that acts on the endothelin A (ET_A) receptor. The dose-response and time-course of the dilator effect of the ET_A receptor antagonist, BQ-123, was investigated in the forearm of healthy volunteers.

Methods Forearm blood flow was measured using mercury-in rubber strain gauge venous occlusion plethysmography.

Results Following intra-arterial infusion of BQ-123 (50 nmol min⁻¹) for 5 min, forearm blood flow increased by approximately 60% over the next 60 minutes; lower doses were without significant effect. The degree of dilatation was similar to that observed in previous studies using 20-fold larger doses.

Conclusions This study confirms that basal endothelin-1 has a role in the physiological regulation of vascular tone. It is possible that at low doses, BQ-123 might be a more sensitive pharmacological tool for the detection of abnormal endothelin-1 mediated constriction.

Keywords: human, endothelin, BQ-123

Introduction

Endothelin-1 (ET-1) is a potent endothelium-derived vasoconstrictor peptide [1] and has been implicated in the regulation of vascular tone in cardiovascular disease [2]. The peptide, BQ-123, is a selective antagonist of endothelin A receptors (ET_A) and blocks constriction to ET-1 in a variety of vessels *in vitro* and *in vivo* [3]. In addition, BQ-123 reverses established contractions to ET-1, indicating that this antagonist can displace endothelin-1 from the ET_A receptor [4, 5]. Recently, it has been demonstrated that local infusion of BQ-123 into the brachial artery causes vasodilatation of the forearm arterial bed of healthy volunteers, suggesting that there is a basal constrictor tone produced by continuous local generation of ET-1 [6]. Vasoconstriction induced by ET-1 (5 pmol min⁻¹) was abolished by co-infusion of BQ-123 (100 nmol min⁻¹). Infusion of this dose of BQ-123 alone for 60 min caused progressive vasodilatation with an increase in forearm blood of approximately 60% [6].

Despite these observations in the human forearm, the results of animal studies have been contradictory, with most studies in healthy animals failing to demonstrate a dilator or hypotensive effect of BQ-123 or other ET_A receptor antagonists [7]. In addition, the doses of BQ-123 that reversed the constrictor response to ET-1 in the human studies were much greater than the predicted dose requirements based on the relative potencies of ET-1 and BQ-123 determined from *in vitro* studies. In human isolated resistance arteries *in vitro*, a 10-fold greater concentration of BQ-123

is needed to reverse ET-1 induced constrictions [5], whereas in the studies *in vivo*, a 20 000-fold excess of BQ-123 was used. The aim of this study was to establish in more detail the dose- and time-dependency of the vasodilator response to BQ-123 in the human forearm.

Methods

The study was approved by the local Ethics Committee and performed using healthy volunteers. Fourteen studies were carried out in 11 subjects (seven males and four females; mean age 25 ± 2 years), none of whom smoked or took any medication. All participants gave informed consent before the study. Subjects lay in a supine position, in a quiet room maintained at a constant temperature of 26°C. A 27 SWG needle was inserted into the brachial artery of the non-dominant arm under local anaesthesia (1% lignocaine) and physiological saline or drugs were infused continuously at a rate of 0.5 ml min⁻¹. Forearm blood flow was measured (ml min⁻¹ 100 ml⁻¹ forearm) simultaneously in both arms using mercury-in-rubber strain gauge venous occlusion plethysmography as described previously [8]. Measurements of flow were made for 5 min every 10 min. The endothelin antagonist BQ-123, (cyclo[-D-aspartyl-L-proline-D-valyl-L-leu-D-thr]; American Peptide Company, Sunnyvale, CA, USA) was dissolved in sterile 0.9% (w/v) saline and passed through a 'Millipore' 0.2 µm bacterial filter immediately before use. Blood flow was measured for 20 min after insertion of the needle, after which BQ-123 was administered as a single dose at 10 (n = 4), 25 (n = 5) or 50 pmol min⁻¹, (n = 5). Each infusion lasted for 5 min and forearm blood flow was measured for a further 60 min, during which saline alone was infused.

Correspondence: Dr R. J. MacAllister, Centre for Clinical Pharmacology, Cruciform Project, University College London, The Rayne Institute, 5 University Street, London WC1E 6JJ.

Calculations and statistics

Forearm blood flow ($\text{ml min}^{-1} 100 \text{ ml}^{-1}$ forearm) was expressed as the ratio of flow in the infused to the control arm. The response to BQ-123 was expressed as a percentage change of this ratio compared with the control period and analysed by calculating the area under the response-time curve (AUC; expressed in arbitrary units) for each dose infused. All results are expressed as mean $\pm$ s.e. mean or 95% confidence intervals (ci) as appropriate and were compared using analysis of variance (ANOVA) where $P < 0.05$ was considered statistically significant.

Results

Infusion of BQ-123 at a dose of 50 nmol min^{-1} for 5 min caused a slowly developing (over 20 min) increase in blood flow in the infused arm that was still present 60 min after the infusion of BQ-123 had stopped (Figure 1). The AUC values for the 20 min control period (1868 ± 186 ; 95% CI 1352–2386) was significantly lower than the AUC values for each of the first, second and third 20 min periods after the infusion stopped (2571 ± 128 (95% CI 2215–2928), 3035 ± 219 (95% CI 2427–3643) and 3123 ± 63 (95% CI 2949–3298) units respectively; $P < 0.05$ by ANOVA for each). Lower doses of BQ-123 did not cause significant changes in forearm blood flow (Figure 1). There were no changes in blood flow in the non-infused arm (data not shown).

Discussion

This study confirms previous work indicating that BQ-123, a potent antagonist of ET-1 at the ET_A receptor, causes

vasodilatation in the human forearm. Local infusion of BQ-123 (50 nmol min^{-1} for 5 min) increased basal forearm blood flow by approximately 60%; lower doses had no effect. The dilatation caused by a 5 min infusion of BQ-123 developed slowly and lasted for up to 1 h after the infusion had stopped, when the concentration of BQ-123 in the forearm would have been negligible.

ET-1 is the most potent endogenous vasoconstrictor substance yet described and its prolonged vasoconstrictor effects are mediated mainly by the ET_A receptor and due to prolonged occupancy of this receptor sub-type [9]. BQ-123 is a cyclic pentapeptide and an ET_A receptor antagonist with a high degree of specificity. In isolated vessels *in vitro* and animals *in vivo*, BQ-123 inhibits the vasoconstriction induced by ET-1. However, most [10–12] but not all [13] studies using BQ-123 or other endothelin antagonists have demonstrated little hypotensive effect in healthy animals when used alone, although a hypotensive effect has been documented in certain animal models of hypertension [10]. These inconsistencies make it difficult to be certain whether endothelin-mediated constriction has a physiological role in the regulation of basal vascular tone in animals, although it might be important in disease states.

In humans however, local infusion of BQ-123 has been shown to dilate the forearm vascular bed of healthy subjects, supporting a role for endothelin in the control of resting arteriolar tone and blood pressure in humans [6]. When infused for 60 min, BQ-123 ($100 \text{ nmol min}^{-1}$) dilated the forearm vascular bed by approximately 60%; a similar dilatation was observed in the present study using a 24-fold smaller dose (50 nmol min^{-1} for 5 min), with no effect seen at lower doses. The time course of the effects of BQ-123 (slow onset dilatation over 20 min, persisting for 30–60 min after the infusion had stopped) was also similar to a previous study using larger doses of BQ-123 (although in the present study the dilatation appeared to be more persistent [6]). The time-course of the dilatation caused by BQ-123 is unlikely to be accounted for by the kinetics of ET-1 displacement from the ET_A receptor, but might depend on the kinetics of the second messengers for ET-1. It is also possible that these doses of BQ-123 (causing relatively high local concentrations) might have other effects (including binding to ET_B receptors [14]) which might contribute to the effects seen. To address this possibility, further studies would be needed to compare the potency of BQ-123 on exogenous ET-1-mediated constriction with its potency as a dilator when infused alone.

In summary, these studies indicate that low doses of BQ-123 dilate the forearm resistance bed of healthy subjects and that the time-course of the dilatation is unlikely to be explained solely by displacement of ET-1 from its receptor. These observations have possible implications for the design of future studies using BQ-123 (or other ET_A receptor antagonists) to investigate endogenous endothelin-mediated contraction in disease states. Previous studies have used large doses of BQ-123 to determine whether the effects of maximal inhibition of ET-1-mediated constriction are abnormal in disease states. From these results it is possible that lower doses might also be useful to detect differences in the threshold or time-course of the response to BQ-123 between healthy subjects and patients with cardiovascular disease.

Figure 1 Vasodilatation caused by a 5 min intra-arterial infusion of BQ-123 at 10 nmol min^{-1} ($\triangle$; $n = 4$), 25 nmol min^{-1} ($\blacktriangle$; $n = 5$) and 50 nmol min^{-1} ($\blacksquare$; $n = 5$). Only the highest dose caused significant dilatation of the forearm vascular bed ($P < 0.01$). Results expressed as mean/vertical bar s.e. mean.

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(Received 22 November 1996,
accepted 21 August 1997)