Vasoconstriction to endogenous endothelin-1 is impaired in patients with Type II diabetes mellitus

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Abstract

Endothelin-1 has potent vasoconstrictor and vasopressor actions contributing to basal vascular tone and maintenance of blood pressure acting predominantly through endothelin-A receptors. Endothelin antagonists may be of value in the treatment of hypertension and heart failure. However, the role of endothelin-1 in the regulation of vascular tone and the potential benefits of endothelin antagonists in non-insulin-dependent diabetes mellitus (Type II diabetes) are less clear. Vasoconstriction to exogenous endothelin-1 is impaired in Type II diabetes. The purpose of this study was to determine whether vasoconstriction to endogenous endothelin-1 acting through the endothelin-A receptor is impaired in Type II diabetes. In ten patients with Type II diabetes and nine controls the endothelin-A receptor antagonist BQ123 was infused intraarterially at 100 nmol/min for 60 min followed by normal saline for 30 min. Forearm blood flow was measured using venous occlusion plethysmography. Control subjects showed gradual onset of vasodilation in response to BQ123 (P < 0.001). Diabetic subjects, however, showed no significant response (P > 0.05). There was a significant difference between the diabetic and control groups (P < 0.05). Blockade of the endothelin-A receptor is associated with impaired vasodilation in Type II diabetes indicating vasoconstriction to endogenous endothelin-1 mediated by the endothelin-A receptor is impaired.

Introduction

Endothelin-1 is a 21 amino acid peptide causing potent and prolonged vasoconstriction of the underlying smooth muscle through endothelin-A and endothelin-B receptors. The endothelin-A receptor is the predominate receptor subtype mediating vasoconstriction. This constriction is modulated by endothelin-B receptor-mediated release of nitric oxide and prostacyclin from the overlying endothelium. Endogenous endothelin-1 acting through the endothelin-A receptor contributes to the maintenance of basal vascular tone [1]. In addition endogenous endothelin-1 plays a physiological role in the control of blood pressure [2]. There is evidence that endothelin-1 contributes to the development of essential hypertension. Elevated plasma concentrations of endothelin-1 have been reported [3] and, in addition, increased sensitivity to endothelin-1 in vivo has been demonstrated [4]. A pathogenic role is also emerging for endothelin-1 in the aetiology of congestive heart failure [5–8]. Given the role of endothelin-1 in the pathophysiology of hypertension and congestive heart failure, endothelin antagonists are being developed for the treatment of these conditions.
It is unclear if endothelin antagonists will be of benefit in all patient subgroups. The role of endothelin-1 in the regulation of vascular tone and the potential benefits of endothelin antagonists in patients with Type II diabetes are less clear. In general reduced responsiveness to endothelin-1 has been reported in the blood vessels of diabetic animals [9–12]. We have previously demonstrated that vasoconstrictor responses to exogenous endothelin-1 are impaired in patients with Type II diabetes when compared with controls [13].

We hypothesized that vasoconstrictor responses to endogenous endothelin-1 mediated through the endothelin-A receptor are also impaired. If impaired vasoconstriction to endogenous endothelin-1 was demonstrated this would suggest endothelin antagonists may have limited value in the treatment of hypertension and heart failure in patients with Type II diabetes.

METHODS

Ten male patients with Type II diabetes were studied. Diabetic control was achieved by diet alone or by diet and oral hypoglycaemic agent. Subjects were excluded if they had a history of cardiovascular disease, hypertension, hypercholesterolaemia, smoking, renal impairment or if they were taking drugs acting on the cardiovascular system. Nine healthy male volunteers were recruited as a control group (see Table 1). All subjects underwent medical assessment. In addition, the diabetic subjects were assessed for the presence of microvascular disease. All subjects gave written informed consent. The local ethics committee of the Queen’s University of Belfast approved the study.

Each subject fasted and abstained from alcohol and caffeine containing products for 10 h prior to the study. The studies took place in a temperature-controlled room (24–26 °C) with the subject supine and the arms resting on a support above the level of the heart. Under local anaesthesia (1% lignocaine; Antigen Pharmaceuticals, Roscrea, Ireland) a 20-gauge polyethylene cannula (Vygon leader cath, Ecouen, France) was inserted into the non-dominant brachial artery. Forearm blood flow (FBF) was measured by strain gauge venous occlusion plethysmography. The hand was excluded from the circulation by inflating a wrist cuff to 200 mmHg for 1 min before and during the FBF measurements. An upper arm cuff was inflated to 40 mmHg for 5–10 s to achieve venous occlusion. A mercury-in-silastic strain gauge was coupled to an electronically calibrated plethysmograph (Medasonics model SPG16, CA, U.S.A.). The voltage output was transferred to a Macintosh personal computer (Performa 630, Apple Computer Inc., CA, U.S.A.) with a MacLab analog-to-digital converter and CHART software (v. 3.4.3; AD Instruments, Sussex, U.K.). FBF was measured in both arms using the non-cannulated arm as a control. The mean of five consecutive FBF measurements was taken for statistical evaluation.

Following a period of at least 30 min rest, during which time 0.9% saline was infused, basal FBF was measured. Using a constant rate infuser (Braun Perfusor pump, Melsungen, Germany), the endothelin-A receptor antagonist BQ123 (cyclo[D-Asp-Pri-D-Val-Leu-D-Trp]; Bachem Torrance, CA, U.S.A.) was infused intravenously at 100 nmol/min at a rate of 1 ml/min for 60 min. FBF was measured during the first 5 min of the infusion and at 5 min intervals thereafter until completion of the infusion. Measurements of FBF were then continued at 10 min intervals after completion of the infusion for a further 30 min during which time 0.9% saline was infused.

The dose of BQ123 was chosen to achieve a local concentration in the forearm that was 10-fold greater than the pA2 (measure of the potency of the antagonist) at the endothelin-A receptor [14]. BQ123 was administered locally to avoid modification of direct vascular responses by systemic reflexes. This dose has previously been shown to have no systemic effect on heart rate, blood pressure or FBF [1].

Unless otherwise stated data are expressed as means with 95% confidence intervals (CI).

The clinical and biochemical characteristics of the patient and control groups were compared using an unpaired Student’s t test. Responses to BQ123 were analysed using summary measures [15] with a paired Student’s t test to compare basal and mean response for within-group dose effects and an unpaired Student’s t test to compare mean change in FBF from baseline for between-group differences.

RESULTS

Fasting blood sugar was significantly higher in the diabetic group (P < 0.01). No other significant differences were found in the clinical and biochemical characteristics given in Table 1 (P > 0.05). There was no difference in plasma endothelin-1 between the diabetic and control subjects (0.8, 95% CI 0.6–1.0 versus 0.7, 95% CI 0.6–0.8; P > 0.05; Table 1). Three of the diabetic subjects had evidence of peripheral neuropathy, and one of these also had background retinopathy and microalbuminuria. One other patient had microalbuminuria. Basal FBF did not differ significantly between the diabetic and control groups (P > 0.05; Table 1). Mean FBF compared with baseline did not change significantly in the control arm in response to BQ123 infused into the contralateral arm (3.3, 95% CI 2.5–4.1 versus 3.6, 95% CI 2.6–4.6 for control subjects; P = 0.44 and 3.4, 95% CI
Table 1 Characteristics of diabetic and control groups

<table>
<thead>
<tr>
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<th>Diabetic</th>
<th>Control</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>54 (range 38–68)</td>
<td>52 (range 38–65)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>30.0 (25.2–34.9)</td>
<td>26.7 (24.3–29.0)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>129 (115–143)</td>
<td>133 (122–144)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77 (71–83)</td>
<td>79 (71–87)</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>88 (80–96)</td>
<td>95 (89–100)</td>
</tr>
<tr>
<td>Microalbuminuria (µg/min)</td>
<td>11.6 (5.1–18.1)</td>
<td>–</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.1 (4.8–5.4)</td>
<td>5.0 (4.5–5.5)</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>12.7 (10.7–14.7)</td>
<td>5.2 (4.7–5.6)</td>
</tr>
<tr>
<td>Glycosylated haemoglobin (HbA₁) (%)</td>
<td>10.0 (8.6–11.5)</td>
<td>6.2 (5.9–6.2)</td>
</tr>
<tr>
<td>Basal FBF (ml·100 ml⁻¹·min⁻¹)</td>
<td>3.4 (2.4–4.4)</td>
<td>3.3 (2.5–4.1)</td>
</tr>
<tr>
<td>Control arm</td>
<td>3.6 (2.5–4.6)</td>
<td>3.2 (2.3–4.0)</td>
</tr>
<tr>
<td>Experimental arm</td>
<td>0.8 (0.6–1.0)</td>
<td>0.7 (0.6–0.8)</td>
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DISCUSSION

We have demonstrated a vasodilator response to endothelin-A receptor antagonism in the forearm arterial vasculature of healthy volunteers. This confirms findings from several previous studies involving human subjects indicating endothelin-1 contributes to basal vascular tone [1,16,17]. We have demonstrated that endothelin-A receptor antagonism does not cause significant vasodilation in patients with Type II diabetes and that responses are blunted in the diabetic group when compared with healthy control subjects. This indicates vasoconstriction mediated by endogenous endothelin-1 is impaired. This is in keeping with previous studies examining the effect of exogenous endothelin-1 both in
\textit{vitro} in animal models of diabetes [9–12] and \textit{in vivo} in patients with Type II diabetes [13].

Given that there was no difference in mean change in FBF from baseline in the control arm during the BQ123 infusion between the diabetic and healthy subjects, indicates that the difference observed in the experimental arm was not simply a time effect.

There are several possible explanations to account for this impaired response. The finding that responses to endothelin-A receptor antagonism are impaired suggests endothelin-A receptor down-regulation. Elevated endothelin-1 levels could result in continuous receptor activation and subsequent endothelin-A receptor down-regulation. Plasma endothelin-1 levels were similar in the diabetic and control subjects suggesting that the proposed receptor down-regulation is not due to increased endothelin-1 levels. The finding that plasma endothelin-1 was normal in the diabetic subjects could be due to the lack of detectable macrovascular disease in our patient group which is known to be associated with elevated plasma endothelin-1 [18]. However, it should be noted that studies have been equivocal with respect to whether or not plasma endothelin-1 is elevated in patients with diabetes [19–22] and it is possible that tissue rather than plasma endothelin-1 is important in determining endothelin-A receptor down-regulation.

Hyperglycaemia has been associated with increased basal FBF [23] and hyperperfusion in the retinal and glomerular microcirculation which is reversible by strict glycaemic control [24]. Reduced contractility to endothelin-1 of bovine pericytes in high concentrations of glucose has been described [9]. It is possible that hyperglycaemia and insulin could cause endothelin receptor down-regulation. Although they have been shown to modify endothelin receptor expression their predominant action is on endothelin-B receptor expression, having no effect on the endothelin-A receptor. It is therefore unlikely that hyperglycaemia or insulin are involved in mediating endothelin-A receptor down-regulation [25,26]. It is possible that hyperglycaemia may result in glycosylation of the endothelin receptor and impair receptor binding; however, impaired contractility to endothelin-1 of bovine pericytes appeared to be unrelated to receptor binding [9]. Alternatively hyperglycaemia may impair intracellular events following binding to the endothelin-A receptor.

BQ123 has no inherent contractile action and has no effect on the contractile response induced by various agonists indicating that the response to BQ123 is due to endothelin-1 antagonism rather than a non-specific action of BQ123 itself on the forearm vasculature [14].

Finally, non-specific impairment of smooth muscle constriction in diabetes is unlikely as similar vasoconstriction has been demonstrated in response to non-specific smooth muscle vasoconstrictors in diabetic and control groups [27].

As can be seen, in contrast to the control group (Figure 3), the diabetic group generally has a minimal response or vasoconstricts to BQ123 (Figure 4). Although the numbers are too small to arrive at firm conclusions it is interesting that the patients with microvascular disease appear to have a more normal vasodilator response. Clearly further study in patients with and without microvascular disease is required to determine if there are differences in responses between these groups.

A potential limitation of this study is that measurement of FBF may not reflect changes in the microcirculation, which may also be important. However, in animal models of diabetes reduced responsiveness to endothelin-1 is seen in both the large vessels and the microvasculature [9,10], suggesting changes in responsiveness to endothelin-1 in the forearm vascular bed may reflect changes within the microvasculature. It is important these findings are now confirmed directly on the microcirculation.

In conclusion, vasoconstriction to endogenous endothelin-1 acting through the endothelin-A receptor is
impaired in Type II diabetes. This has implications for the potential benefit of endothelin antagonists in heart failure and hypertension in patients with Type II diabetes. Further studies in diabetic patients with hypertension and heart failure are required to elucidate if endothelin antagonists will be of benefit in this group of patients.

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REFERENCES


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