Endothelin receptor blockade improves endothelial function in human internal mammary arteries

Subodh Verma\textsuperscript{a}, Fina Lovren\textsuperscript{a}, Aaron S. Dumont\textsuperscript{a}, Kieren J. Mather\textsuperscript{a}, Andrew Maitland\textsuperscript{a}, Teresa M. Kieser\textsuperscript{a}, William Kidd\textsuperscript{b}, John H. McNeill\textsuperscript{b}, Duncan J. Stewart\textsuperscript{c}, Christopher R. Triggle\textsuperscript{a}, Todd J. Anderson\textsuperscript{a, \*}

\textsuperscript{a}Faculty of Medicine, The University of Calgary, Calgary, Canada
\textsuperscript{b}Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, Canada
\textsuperscript{c}Faculty of Medicine, The University of Toronto, Toronto, Canada

Received 16 March 2000; accepted 31 July 2000

Abstract

Objective: Endothelial dysfunction, specifically endothelium-derived contracting factors have been implicated in the development of arterial conduit vasospasm. The potent vasoconstrictor endothelin-1 (ET-1) has received much attention in this regard. The present study was designed to evaluate the role of ET-1 in the development of endothelial dysfunction in human internal mammary arteries (IMA). To this aim, we examined the effects of specific and non-specific ET-receptor antagonists on endothelial function (assessed using acetylcholine (ACh)-induced vasodilation) in segments of IMA obtained during coronary artery bypass graft (CABG) surgery.

Methods: Vascular segments of IMA were obtained from 51 patients undergoing elective coronary artery bypass graft (CABG) surgery and in vitro endothelium-dependent and -independent responses to ACh and sodium nitroprusside (SNP) were assessed. Isometric dose response curves (DRC) to ACh and SNP were constructed in pre-contracted rings in the presence and absence of bosentan (ET receptor antagonist, 3 \mu M), BQ-123 (ET\textsubscript{A} antagonist, 1 \mu M) and BQ-788 (ET\textsubscript{B} antagonist, 1 \mu M) using the isolated organ bath apparatus. Percent maximum relaxation (%E\textsubscript{max}) and sensitivity (pEC\textsubscript{50}) were compared between interventions.

Results: ACh caused dose-dependent endothelium-mediated relaxation in IMA (%E\textsubscript{max} 43 \pm 4, pEC\textsubscript{50} 6.74 \pm 0.12). In the presence of bosentan, BQ-123 and BQ-788 ACh-induced relaxation was significantly augmented (%E\textsubscript{max} bosentan 60 \pm 3, BQ-123 56 \pm 4, BQ-788 53 \pm 5 vs. control 43 \pm 4, P<0.05) without affecting sensitivity. The effects of these antagonists were endothelium-specific since endothelium-independent responses to SNP remained unaltered. Furthermore, the beneficial effects were independently and maximally mediated by ET\textsubscript{A} and ET\textsubscript{B} receptors (%E\textsubscript{max} bosentan 60 \pm 3 vs. BQ-123 56 \pm 4 vs. BQ-788 53 \pm 5 vs. control 43 \pm 4, P<0.05).

Conclusions: These data uncover, for the first time, beneficial effects of ET receptor blockade on endothelium-dependent vasorelaxation in human IMA. © 2001 Elsevier Science BV. All rights reserved.

Keywords: Endothelial function; Endothelins; Cardiovascular surgery; Vasoactive agents; Vasoconstriction/dilation

1. Introduction

The endothelium plays a vital role in vascular homeostasis through the release of autocrine and paracrine sub-stances [1,2]. In addition to vasodilation, the endothelium exerts anti-atherogenic effects through potent inhibition of platelet aggregation, smooth muscle proliferation and leukocyte adhesion [1,2]. A growing body of evidence implicates abnormalities in endothelial function as an early and integral mediator of a variety of cardiovascular disease states [2]. Disruption of the critical balance between endothelium-derived relaxing and contracting factors is believed to predispose the vascular smooth muscle to...

This article is referred to in the Editorial by N. Hoogerwerf (pages 15–16) in this issue.
increased tone, decreased vasomotion, altered reactivity, changes in structure/geometry, enhanced platelet aggregation and eventual atherosclerosis / graft failure [2]. Elucidating the mechanism(s) of endothelial dysfunction in bypass conduits has implications in terms of vessel spasm, graft patency and the outcome of coronary artery bypass graft (CABG) surgery.

Accumulating evidence suggests that endothelium-derived vasoconstrictors (thromboxane, prostanoids and endothelin) may represent important mediators of peri-operative spasm in arterial conduits [3–5]. Commendable studies from Schaff’s group [3] have suggested that these vasoconstrictors may be released secondary to hypoxemia. The growing interest in the role of endothelin-1 (ET-1) as a potential arterial spasmogen has been fueled by several distinct lines of evidence. First, bypass conduits react strongly and dose-dependently to ET-1 [6]. Second, ET-1 exaggerates the pressor responses to endogenous constrictors such as norepinephrine [7] and inhibits the actions of endothelium-derived nitric oxide (NO). Third, the plasma levels of ET-1 are elevated in patients undergoing CABG surgery [8] and, fourth, blood vessel ET-1 content is increased in atherosclerotic human internal mammary arteries (IMA) and modulates graft flow in the peri-operative period [9]. Although ET antagonists have been shown to inhibit ET-1 mediated contraction in IMA [10], whether these agents improve endothelial function remains unknown. Given the potent vasoconstrictor, mitogenic and pro-thrombotic actions of ET-1 [11], targeting these effects may uncover novel approaches to the management of acute peri-operative spasm. Additionally, such interventions may serve to impede long-term graft atherosclerosis. We herein describe, for the first time, the endothelial protective effects of ET receptor blockade in IMA from patients undergoing CABG surgery.

2. Methods

2.1. Patient characteristics and risk factors

A total of 51 patients undergoing elective CABG surgery were recruited following written informed consent. The study was approved by the Institutional Review Committee for Scientific and Ethical Standards. The patient characteristics and risk factors are detailed in Table 1.

2.2. Isolated vascular ring experiments

Distal segments of the IMA not used for bypass grafting were placed in oxygenated physiological salt solution and immediately transferred to the pharmacology laboratory (within 30 min). The vessels were cleaned of adherent tissue and cut into rings (~5 mm in length) and suspended in isolated tissue baths (volume 25 ml) containing oxygenated Kreb–Ringer bicarbonate buffer with the following composition (in mmol/l): NaCl (118), KCl (4.7), CaCl₂ (2.5), KH₂PO₄ (1.2), MgSO₄ (1.2), NaHCO₃ (25), dextrose (11.1) and disodium calcium edetate (0.026), maintained at 37°C and bubbled with 95% O₂ and 5% CO₂ as described previously [12]. A progressive resting tension of 2.5 g was applied (determined by preliminary experiments to afford the optimum tension-length relationship). All experiments were performed in the presence of indomethacin (10⁻⁵ mol/l) to prevent the synthesis of vascular prostaglandins. Following equilibration for 90 min, isometric dose response curves (DRC) were recorded using the following protocol: (a) cumulative DRC to phenylephrine (10⁻⁸ to 10⁻⁵), (b) cumulative DRC to acetylcholine (ACh) in rings pre-contraction with the ED₅₀ of phenylephrine, (c) cumulative DRC to ACh in the presence and absence of bosentan (ET₁/β receptor antagonist, 3 μM for 20 min), BQ-123 (ET₄ receptor antagonist, 1 μM for 20 min) and BQ-788 (ET₂ receptor antagonist, 1 μM for 20 min), and (d) cumulative DRC to sodium nitroprusside (SNP) in the presence and absence of ET antagonists. Since ET antagonists bind strongly and often irreversibly to ET receptors, distinct segments were used to evaluate each antagonist. ACh responses in the absence of ET blockade served as the control group. The tissues were allowed to equilibrate between each DRC for 60–90 min. The presence of the endothelium was confirmed in each ring by relaxation to ACh. Bosentan, BQ-123 and BQ-788 are potent endothelin receptor antagonists at the concentrations used [13–15].

All drugs and chemicals were obtained from Sigma (St.

<table>
<thead>
<tr>
<th>Table 1: Demographic characteristics*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
</tr>
<tr>
<td>Obesity (BMI ≥ 27), n (%)</td>
</tr>
<tr>
<td>Family history, n (%)</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
</tr>
<tr>
<td>Total cholesterol (mM)</td>
</tr>
</tbody>
</table>

* Smoking was considered as current smoker or past smoker who quit within 3 months. Hypertension was defined per history and measurement (140/90). All diabetics were on oral hypoglycemics. None were on insulin. Family history included ischemic heart disease, stroke and peripheral vascular disease. Hypercholesterolemia was defined as total cholesterol greater than 6.0 mM.
Louis, MO, USA). Bosentan was a gift from Actelion (Switzerland).

Results are expressed as mean±S.E. Percent maximum relaxation to ACh (%E_{max}) and agonist sensitivity (pEC_{50} = -\log EC_{50}; EC_{50} is concentration that evokes 50% relaxation. Values were determined using non-linear regression) were compared between interventions. ‘n’ represents the number of patients employed in the study. %E_{max} values were compared using a paired two-tailed t-test. The DRC were compared using repeated measures analysis of variance followed by a Newman–Keul’s test for post-hoc comparisons. A P less than 0.05 was considered to be statistically significant.

3. Results

3.1. Patient characteristics

Table 1 depicts the characteristics and risk factor profile of the patient population. The distribution of coronary artery disease risk factors in this study population was comparable to other studies employing similar methodologies [16].

3.2. Vascular relaxation

In pre-contracted arteries, ACh caused dose-dependent endothelium-mediated relaxation (%E_{max} 43±4%, pEC_{50} 6.74±0.12). This effect was endothelium-specific since it was abolished in vessels denuded of the endothelium (by rolling vessels over a metallic probe, data not shown). The effects of endothelin receptor blockade (with bosentan, BQ-123 and BQ-788) on ACh-mediated relaxation are depicted in Figs. 1–4. In the presence of the mixed ET_A/B receptor blocker (bosentan, 3 μM for 20 min), endothelium-mediated relaxation to ACh was improved significantly (%E_{max} 60±3 vs. control, P<0.05) without an effect on agonist sensitivity (pEC_{50} vs. 6.76±0.09 vs. control 6.74±0.12, P<0.05, Fig. 1). Similar endothelial augmenting effects were noted in the presence of the specific ET_A and ET_B receptor antagonists (%E_{max} BQ-123: 56±4 vs. control, P<0.05, Fig. 2; BQ-788 53±5 vs. control, P<0.05, Fig. 3). Furthermore, ACh-induced relaxation was improved to a similar degree with the three ET antagonists (Fig. 4). The beneficial effects were endo-

---

**Fig. 1.** Endothelium-dependent vascular relaxation to ACh in internal mammary arteries in the absence (control) and presence of bosentan (3 μM for 20 min). Data are represented as mean±S.E. Vascular relaxation is expressed as % maximum relaxation in rings pre-contracted with the ED_{50} of phenylephrine. *P<0.05, different from control. Bosentan augments endothelial function in human internal mammary arteries.

**Fig. 2.** Endothelium-dependent vascular relaxation to ACh in internal mammary arteries in the absence (control) and presence of BQ-123 (1 μM for 20 min). Data are represented as mean±S.E. Vascular relaxation is expressed as % maximum relaxation in rings pre-contracted with the ED_{50} of phenylephrine. *P<0.05, different from control.
4. Discussion

4.1. Key observations

The main observations that emanate from this study are (i) endothelin-receptor blockade improves endothelial-mediated vasodilation in human IMA and (ii) the beneficial effects of endothelin antagonism are independently and equally mediated via ET<sub>A</sub> and ET<sub>B</sub> receptors. To our knowledge, this is the first report describing endothelial protective effects of ET antagonists on ACh-mediated vasorelaxation in IMA. Since endothelial dysfunction may contribute towards the development of peri-operative vasospasm [3–5] and graft atherogenicity [1,2], these results may have both acute and chronic implications for CABG surgery.

4.2. IMA, endothelial dysfunction and vasospasm: role of ET-1

The IMA is unequivocally the conduit of choice for coronary revascularization due to its excellent long-term patency [17]. Indeed the resilience of the IMA and its inherent thromboresistance have been attributed, in part, to better endothelial function in these vessels (compared to saphenous veins and other arterial conduits) [18]. However, the small diameter of this arterial conduit predisposes it to acute peri-operative vasospasm which is a critical determinant of postoperative myocardial infarction and death following CABG surgery. Elucidating the mechanism(s) of vasospasm has been an area of intense research and studies by Schaff [3,4] and Lin [5] are widely quoted. Although the definitive mechanisms remain unclear, it appears that endothelial dysfunction, specifically the release of thromboxane A<sub>2</sub> and ET-1 may play a significant role [3,4,19]. In addition to directly causing vascular smooth muscle contraction (via interaction with thromboxane and ET receptors on smooth muscle) these agents can impair endothelial function and vascular reactivity through inhibition of NO production/release [2]. Studies have suggested that the release of endothelium-derived vasoconstrictors may be closely associated with the expression of hypoxemia [3,5], a commonly observed peri-operative complication. Factors such as low cardiac output, intrapulmonary blood shunting, hypoventilation and technical errors may evoke a potent hypoxic response in the peri-operative period [5] and predispose to arterial spasm via production of thromboxane A<sub>2</sub>. Whether such a mechanism underlies the production of ET-1 in IMA is unknown.
Another possibility is that cardiopulmonary bypass (with the associated inflammatory and cytokine response) may result in the generation of potent vasoconstrictors and predispose to arterial spasm. We are currently investigating this hypothesis. Whatever the exact mechanism, it is apparent that counteracting the actions of these vasoconstrictors may serve to restore endothelial function and vascular reactivity in IMA.

4.3. Endothelin antagonists and endothelial function in IMA: the present study

In this study we have demonstrated that ET receptor blockade improves endothelial function in human IMA. These data are important since they uncover an improvement in ACh (and hence NO)-mediated vasodilation in response to ET antagonism. Although previous studies have examined the effects of ET receptor blockers on IMA vascular function, they have focussed on the ability of such antagonists to counteract the contraction elicited by graded doses of ET-1 [10]. ACh-mediated vasorelaxation has not been previously assessed in response to acute ET receptor blockade.

A brief discussion of the potential mechanism(s) of endothelial protection by ET blockers deserves mention. As highlighted earlier, endothelial dysfunction can be viewed as the net balance of endothelium-derived vasoconstriction and vasodilation; derangements in either segment (or both) may predispose to increased tone and eventual vasospasm [2]. Therefore, the beneficial effects noted in the present study may be due to (i) antagonism of ET-1 action on vascular smooth muscle ET receptors, (ii) improvement in NO-mediated vasodilation (by release of tonic inhibition of ET-1 on NO production/release) or (iii) a combination of the above mechanisms. Alternatively, it may be hypothesized that decreased endothelium-dependent relaxation in IMA is secondary to diminished NO production and that ET receptor blockade improves endothelial function merely by restoring the balance of NO/ET-1. Although this is an attractive proposition, evidence suggests that the l-arginine NO system is well preserved in human IMA [18–21]. Furthermore, depressed endothelium-dependent relaxation in these vessels is not related to increases in superoxide production, deficiencies in l-arginine, tetrahydrobiopterin or reduced membrane fluidity [16]. These data provide indirect evidence for a primary role of vasoconstrictors in the development of endothelial dysfunction in IMA. Studies documenting increased ET-1 levels in segments of human IMA [9] lend further credence to this concept.

The present data raise an important question regarding the involvement of ET\textsubscript{A} versus ET\textsubscript{B} receptors in mediating endothelial protection. Conventional, it is believed that the actions of ET-1 are facilitated through ET\textsubscript{A} and ET\textsubscript{B} receptors, the former mediating the majority of the vasoconstrictor effects (on smooth muscle) and the latter interacting with endothelium-derived NO to induce vasodilation (transient) [11]. In IMA, Pate et al. [22] have demonstrated that ET-1 mediates its effects predominately through ET\textsubscript{A} receptors with lesser contribution from ET\textsubscript{B} receptors. This would imply that ET receptor blockade with BQ-123 would improve endothelial function to a greater extent than BQ-788 which was not the case in the present study. As correctly pointed out by Pate et al. the results obtained are from patients undergoing CABG surgery with multiple risk factors and damaged vessels to begin with, hence these data may differ from those obtained in healthy vessels. The patient demographics in the study by Pate et al. are not available and hence a direct comparison of the two studies is not feasible. In other studies, pharmacological characterization of receptor subtypes in IMA suggest that both ET\textsubscript{A} and ET\textsubscript{B} mediate the ET-1-induced contraction [10,21] and that the endothelium of the arteries does not express ET\textsubscript{B} receptors linked to NO production [10]. Indeed, the functional significance of these observations is strengthened by the current data demonstrating that both ET\textsubscript{A} and ET\textsubscript{B} receptors improve endothelial function independently (and to a similar degree) when compared to the mixed ET\textsubscript{A/B} blocker bosen-tan. This is the first report depicting an equal contribution of ET\textsubscript{A} and ET\textsubscript{B} receptors towards endothelial dysfunction in IMA. The observation that combined ET\textsubscript{A/B} receptor blockade (with bosentan) did not result in an additive/synergistic effect may suggest that antagonism of ET\textsubscript{A} or ET\textsubscript{B} receptors (individually) exerts maximal endothelial protection. In addition, results from the present study were obtained in the presence of indomethacin suggesting that blockade of prostaglandin synthesis may be required to elicit the protective effects noted.

4.4. Limitations

Given the patient demographics, the contribution of individual risk factors towards endothelial dysfunction and the observed effects of ET receptor blockade cannot be assessed. All major risk factors for atherosclerotic vascular disease have been associated with impaired NO activity [23]. Hence it is plausible that the beneficial effects of ET antagonism may extend non-specifically to any process that dampens endothelial function through affecting NO production, release or availability. Unfortunately, the study population undergoing bypass surgery has a clustering of CAD risk factors. Hence to find patients with one isolated risk factor undergoing bypass surgery without other risk factors, such as hypertension, smoking, obesity, hyperlipidemia, family history etc., is extremely difficult. In addition finding patients just on angiotensin converting enzyme inhibitors or beta-blockers without any other risk factors would require us to screen ~500–700 patients. Furthermore, it is impossible to discontinue medication usage of these patients prior to CABG surgery. Of the 22 patients on ACEI, 16 were on \beta-blockers, five on endo-
5. Conclusion

In conclusion, results from the present study demonstrate, for the first time, that ET receptor blockade improves endothelial function in human IMA. Furthermore, they reveal that the beneficial effects are mediated, independently and maximally via ET_{A} and ET_{B} receptors. Understanding and improving endothelial function may promote thromboresistance, decrease peri-operative vasospasm and impede IMA atherosclerosis.

Acknowledgements

This study was supported by the Heart and Stroke Foundation of Canada (T.J.A., C.R.T., J.H.McN.) and the Medical Research Council of Canada (C.R.T., J.H.McN.). Subodh Verma, MD, PhD is a Fellow of the Medical Research Council of Canada, Heart and Stroke Foundation of Canada and Alberta Heritage Foundation for Medical Research (AHFMR). K.M. is an AHFMR Clinical Fellow. A.S.D. is the recipient of a studentship award from AHFMR. Dr T.J.A. is a Clinical Investigator of the AHFMR. We thank Dr. M. Clozel for constructive criticism of the manuscript and for the generous gift of bosentan (Actelion Ltd, Switzerland). Subodh Verma was the International Society of Heart Research Young Investigator Award finalist for this research (2000).

References