Endothelin-A Receptor Antagonism Reduces Blood Pressure and Increases Renal Blood Flow in Hypertensive Patients With Chronic Renal Failure

A Comparison of Selective and Combined Endothelin Receptor Blockade

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Background—Endothelin (ET) is implicated in the pathophysiology of chronic renal failure (CRF). We therefore studied the systemic and renal hemodynamic effects of ET receptor antagonists in CRF and examined differences between selective ETA, selective ETB, and combined ETA/B receptor blockade.

Methods and Results—We conducted a randomized, placebo-controlled, double-blind, 4-way crossover study comparing selective ET receptor antagonists BQ-123 (ETA) and BQ-788 (ETB), given alone and in combination, in acute studies in 8 hypertensive CRF patients and 8 matched healthy controls. BQ-123, alone and in combination with BQ-788, reduced blood pressure in CRF, particularly with BQ-123 alone (mean arterial pressure: controls 4, CRF 13, \( P < 0.01 \) versus placebo). In CRF, in the face of this fall in blood pressure, BQ-123 substantially increased renal blood flow (38.8, \( P < 0.01 \) versus placebo) and reduced renal vascular resistance (44.5, \( P < 0.01 \) versus placebo) when given alone but not when combined with BQ-788. These changes were accompanied by a reduction in effective filtration fraction. BQ-123, alone or in combination with BQ-788, had minimal effects on the renal circulation in healthy controls, and BQ-788 alone produced both systemic and renal vasoconstriction in CRF and healthy controls.

Conclusions—ETA receptor antagonism was highly effective in lowering blood pressure in CRF patients currently treated for hypertension. In addition, there were effects consistent with a renoprotective action. However, because the ETB receptor appears to play a key role in the maintenance of tonic renal vasodilation, combined ETA/B receptor antagonism, although it lowered blood pressure, did not confer these renal benefits. (Circulation. 2004;109:1186-1193.)

Key Words: blood flow ■ blood pressure ■ endothelin ■ kidney ■ hemodynamics

The endothelins are powerful vasoconstrictor peptides, of which endothelin-1 (ET-1) is the major isoform. It is produced by vascular endothelium and acts through 2 receptors in the blood vessels to modulate vascular tone. Endothelin-A (ETA) and endothelin-B (ETB) receptors are found in vascular smooth muscle, where their activation mediates vasoconstriction. ETB receptors are also found on vascular endothelium, where their activation promotes vasodilation through the release of nitric oxide and prostaglandins. Additionally, vascular ETB receptors act as a major source of ET-1 clearance from the circulation and renal tubular ETB receptors may contribute to natriuresis. Studies in humans show the importance of ET-1 in the maintenance of vascular tone and blood pressure and suggest therapeutic potential for endothelin receptor antagonists in pathophysiological states characterized by vasoconstriction, such as essential hypertension and pulmonary arterial hypertension.

Chronic renal failure (CRF) is characterized by systemic and renal vasoconstriction and commonly associated with hypertension. Given that endothelin receptor antagonists cause vasodilation and lower blood pressure and ameliorate renal dysfunction in experimental models of kidney disease, they may be useful in the treatment of CRF. The aim of the present study was to show whether endothelin receptor antagonists would produce beneficial systemic or renal hemodynamic effects in CRF. Because of the potential opposing effects at ETA and ETB receptors, we directly compared the effects of regimens involving ETA and ETB.
receptor blockade separately and in combination in CRF patients and matched healthy controls.

Methods

Subjects

We aimed to recruit 8 men with stable CRF and 8 matched healthy controls to the studies, which were performed in the University of Edinburgh’s Clinical Research Centre with the approval of the local research ethics committee and the written informed consent of each subject. The investigations conformed to the principles outlined in the Declaration of Helsinki.

For 3 days before each study, subjects adhered to a standard diet containing 150 mmol of sodium. All subjects abstained from alcohol, nicotine, and caffeine-containing products for 24 hours and had a light breakfast before attending on each study day. All studies were performed in a quiet, temperature-controlled room at 22°C to 24°C, with the subject recumbent throughout, except when voiding urine.

Healthy subjects who had taken any medications in the previous 2 weeks were excluded from the study. Patients continued taking their normal medication up to and including each study day with the exception of diuretics, which they omitted that morning. To enhance homogeneity and avoid other influences on vascular reactivity, patients with vasculitis, other systemic inflammatory disease, polycystic kidney disease, nephrotic syndrome, or obstructive uropathy were excluded. Additionally, patients with significant comorbid disease, including diabetes mellitus, heart or lung disease, peripheral vascular disease, or hypercholesterolemia, were excluded. The 2 study groups were matched for age, weight, serum cholesterol, and blood pressure (Table 1).

Drugs

BQ-123 (Clinalfa AG), a selective ETA receptor antagonist, was infused at 100 and 1000 nmol/min for 15 minutes at each dose. These doses were selected from a previous study as having a threshold and maximum hemodynamic effect in healthy controls. BQ-788 (Clinalfa), a selective ETB receptor antagonist, was infused at 30 and 300 nmol/min for 15 minutes, doses shown to be hemodynamically active in a previous systemic dose-ranging study. Drugs were dissolved in physiological saline (0.9%; Baxter Healthcare Ltd) and infused intravenously at a constant rate of 1 mL/min. Saline was administered as placebo.

Para-aminohippurate sodium (PAH; Clinalfa) and inutest (Fresenius Pharma, Austria GmbH) were dissolved in dextrose 5% (Baxter) and administered as a bolus loading dose of 0.4 g of PAH and 3.5 g of inutest in 100 mL of dextrose over 15 minutes and a maintenance infusion of 6.6 g/L PAH and 10 g/L inutest at a rate of 2 mL/min. For subjects with a calculated glomerular filtration rate (GFR) <40 mL/min, doses of PAH and inutest were reduced by one third.

Assays

At prespecified time points, venous blood was collected into EDTA tubes (Sarstedt) for measurement of PAH, inulin, and hematocrit (Hct), plasma ET-1, plasma renin activity, angiotensin II, and aldosterone, and into plain tubes (Sarstedt) for the measurement of serum sodium. Additionally, 20-mL aliquots of urine from each voiding were collected into plain tubes for the measurement of urinary PAH, inulin, sodium, and protein.

Hct was measured on whole blood with a Coulter counter. All other blood samples were centrifuged immediately at 1000g at 4°C for 20 minutes, and plasma and urine were stored in plain tubes at −80°C. Inulin was determined by spectrophotometry after hydrolysis to fructose, and PAH and BQ-123 were determined by high-performance liquid chromatography. BQ-788 assay was not sufficiently sensitive for its detection in plasma. Urinary and plasma sodium concentrations were measured by flame photometry. ET-1, angiotensin II, plasma renin activity, and aldosterone were determined by radioimmunoassay.

Study Protocol

This was a randomized, double-blind, placebo-controlled study. Subjects attended for 4 visits, receiving placebo, BQ-123, BQ-788, or the combination of BQ-123 and BQ-788. Because previous studies with the same doses of BQ-123 or BQ-788 have demonstrated that hemodynamic changes return to baseline after 4 hours, each visit was separated by 7 days to ensure complete washout of the study drugs. An individual otherwise unconnected with the study prepared the drugs to maintain blinding. On each study day, an 18 standard wire gauge cannula was sited in an antecubital vein in each arm. Diuresis was induced by 500 mL of 5% dextrose over 30 minutes through the left arm cannula. After 15 minutes, loading doses of PAH and inutest were administered through the same cannula. Thereafter, maintenance infusions of PAH and inutest and 5% dextrose at 260 mL/h continued throughout the study. Blood pressure, cardiac output and heart rate were recorded by validated noninvasive automated techniques every 15 minutes, and urine was collected every 30 minutes by spontaneous voiding. After a 2-hour equilibration period, baseline measurements were made over 2 30-minute urine-collection periods. The low dose of antagonist was then administered through the right antecubital cannula, followed by 3 30-minute collection periods. The higher dose of antagonist was then administered, followed by 5 further 30-minute collection periods.

At the midpoint of each collection period, blood was sampled from the right antecubital cannula for PAH, inulin, sodium, and Hct. At 0, 60, and 90 minutes after the start of low- and high-dose antagonist and at the end of the study, additional samples were taken for plasma hormone measurements. BQ-123 was measured before and at 15, 45, and 90 minutes after the start of each dose of antagonist and at the end of the study.
Data Analysis
Data were stored and analyzed with Microsoft Excel (version 5.0, Microsoft Ltd). Demographic data are expressed as mean±SEM, and comparisons between groups were examined by unpaired Student’s t tests. Blood pressure at each time point was calculated as the mean of 2 recordings and represented as mean arterial pressure (MAP=diastolic blood pressure+1/3 pulse pressure). Bioimpedance data at each time point were calculated as the mean of 4 recordings, each the average of 15 consecutive heart beats. Data were corrected with body surface area to give cardiac index for direct comparison of subjects.

Systemic vascular resistance index (SVRI) was calculated by dividing MAP by cardiac index and expressed in dyne·s·m⁻²·cm⁻³. Effective renal blood flow (ERBF) was calculated by dividing MAP by cardiac index and expressed in dyne·s·m⁻²·cm⁻³. GFR and effective renal plasma flow (ERPF) were calculated from plasma inulin and PAH clearances, respectively. Effective renal plasma flow rate and fractional excretion as urinary sodium (UNaV, mmol/min) were calculated as urinary sodium (equiv. of NaCl in mmol) divided by urinary creatinine concentration (mg/dL) × urine volume (mL/day) × 0.01803.

Urinary sodium excretion was calculated as urinary sodium (equiv. of NaCl in mmol) divided by urinary creatinine concentration (mg/dL) × urine volume (mL/day) × 0.01803. Plasma aldosterone, pg/mL, was calculated as plasma aldosterone concentration (ng/L) divided by plasma creatinine concentration (mg/dL) × 0.01803.

Results
Twelve renal patients were recruited; 1 developed nausea after receiving BQ-123, and 1 was unable to void urine at 30-minute intervals. Two withdrew for reasons unrelated to the study. Eight patients and all healthy controls completed all phases of the study without adverse events. The renal diagnosis and concomitant medication taken by the patients who completed the study are given in Table 2. Baseline parameters are shown in Table 3.

Systemic Hemodynamics
In CRF patients, placebo was associated with increases in SVRI to study end (3478±240 versus 3797±301 dyne·s·m⁻²·cm⁻³, P<0.05) and MAP (100.7±3.8 versus 108.0±4.1 mm Hg, P<0.01), consistent with the waning effects of antihypertensive medication (Figures 1 and 2).

Table 2. Renal Patients: Diagnosis and Medications

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cause of Renal Impairment</th>
<th>Drugs Used</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>IgA nephropathy</td>
<td>Enalapril</td>
</tr>
<tr>
<td>2</td>
<td>IgA nephropathy</td>
<td>Ranitidine</td>
</tr>
<tr>
<td>3</td>
<td>IgA nephropathy</td>
<td>Enalapril, doxazosin, bicarbonate, omeprazole</td>
</tr>
<tr>
<td>4</td>
<td>IgA nephropathy/HSP</td>
<td>Labetalol</td>
</tr>
<tr>
<td>5</td>
<td>Proliferative GN</td>
<td>Fosinopril, atenolol</td>
</tr>
<tr>
<td>6</td>
<td>Proliferative GN</td>
<td>Enalapril, metoprolol, nifedipine, allopurinol, frusemide</td>
</tr>
<tr>
<td>7</td>
<td>Renal calculi, single kidney</td>
<td>Enalapril, bicarbonate, 1-α calcidol</td>
</tr>
<tr>
<td>8</td>
<td>Not known; late presentation</td>
<td>Lisinopril, bicarbonate, omeprazole, frusemide</td>
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HSP indicates Henoch-Schönlein purpura; GN, glomerulonephritis.

<table>
<thead>
<tr>
<th>TABLE 3. Baseline Data</th>
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<tr>
<td></td>
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<tr>
<td>MAP, mm Hg</td>
</tr>
<tr>
<td>SVRI, dyne·s·m⁻²·cm⁻³</td>
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<tr>
<td>Cardiac index, L·min⁻¹·m⁻²</td>
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<tr>
<td>Heart rate, bpm</td>
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<tr>
<td>ERBF, mL·min⁻¹</td>
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<tr>
<td>ERVR, mm Hg·min⁻¹·L⁻¹</td>
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<tr>
<td>EFF, %</td>
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<tr>
<td>GFR, mL·min⁻¹·1.73 m⁻²</td>
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<tr>
<td>UNaV, μmol/min</td>
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<tr>
<td>Urinary protein, mg/L</td>
</tr>
<tr>
<td>Plasma ET-1, pg/mL</td>
</tr>
<tr>
<td>PRA, pg·mL⁻¹·h⁻¹</td>
</tr>
<tr>
<td>Plasma ANG II, pg/mL</td>
</tr>
<tr>
<td>Plasma aldosterone, pg/mL</td>
</tr>
</tbody>
</table>

NS indicates not significant; UNaV, urinary sodium excretion; PRA, plasma renin activity, and ANG II, angiotensin II.

Values are given as mean of 2 baseline periods over the 4 study days ±SEM (range).

*Values below limit of laboratory assay.
†n=7; 1 value below limit of detection.
SVRI was reduced to a similar extent by BQ-123 and BQ-123/788 (BQ-123 \(-630\pm145 \text{ dyne \cdot s \cdot m}^{-2} \cdot \text{cm}^{-3}\), BQ-123/788 \(-617\pm158 \text{ dyne \cdot s \cdot m}^{-2} \cdot \text{cm}^{-3}\), both \(P<0.01\) versus placebo). MAP was reduced in CRF patients after BQ-123/788 (7.4\pm1.6 mm Hg, \(P<0.01\) versus placebo) but to a greater extent after BQ-123 alone (BQ-123 \(-12.9\pm1.7\) mm Hg, \(P<0.01\) versus placebo and BQ-123/788). Systemic hemodynamic responses to endothelin receptor antagonism showed no correlation with baseline blood pressure.

In healthy controls, placebo did not alter systemic hemodynamics. Both BQ-123 and BQ-123/788 reduced SVRI to a similar extent, equivalent to that seen in CRF (BQ-123 \(-591\pm104 \text{ dyne \cdot s \cdot m}^{-2} \cdot \text{cm}^{-3}\), BQ-123/788 \(-498\pm159 \text{ dyne \cdot s \cdot m}^{-2} \cdot \text{cm}^{-3}\), both \(P<0.01\) versus placebo). The reductions in MAP after BQ-123 and BQ-123/788 were equal (BQ-123 \(-3.6\pm1.4\) mm Hg, BQ-123/788 \(-3.7\pm1.6\) mm Hg, both \(P<0.01\) versus placebo) and less than those seen in CRF (\(P<0.05\)). BQ-788 increased MAP (CRF \(7.0\pm1.6\) mm Hg, healthy controls \(5.8\pm1.5\) mm Hg, both \(P<0.01\) versus placebo) and SVRI (CRF \(454\pm114 \text{ dyne \cdot s \cdot m}^{-2} \cdot \text{cm}^{-3}\), \(P<0.01\) versus placebo; healthy controls \(390\pm76 \text{ dyne \cdot s}^{-1} \cdot \text{m}^{-2} \cdot \text{cm}^{-3}\), \(P<0.05\) versus placebo) to a similar extent in CRF patients and controls.

Renal Hemodynamics

In CRF patients, BQ-123 but not BQ-123/788 produced striking increases in ERBF and reductions in ERVR and EFF (ERBF \(102\pm74 \text{ mL/min}\), ERVR \(243\pm91 \text{ mm Hg} \cdot \text{min} \cdot \text{L}^{-1}\), EFF \(4.2\pm2.9\%\); all \(P<0.01\) versus placebo and BQ-123/788). GFR did not change. By contrast, in healthy controls, BQ-123 and BQ-123/788 were neutral with respect to ERBF, ERVR, EFF, and GFR. (See Figures 2 and 3.)

In both groups, BQ-788 reduced ERBF (CRF \(-77\pm72 \text{ mL/min}\), healthy controls \(-134\pm47 \text{ mL/min}\); both \(P<0.05\) versus placebo) and increased ERVR (CRF \(112\pm63 \text{ mm Hg} \cdot \text{min} \cdot \text{L}^{-1}\), healthy controls \(39\pm12 \text{ mm Hg} \cdot \text{min} \cdot \text{L}^{-1}\); both \(P<0.05\) versus placebo). These changes were apparent even
at the low dose and were associated with a reduction in GFR and an increase in EFF.

**Urinary Sodium Excretion and Protein Excretion**

No changes in sodium excretion or fractional excretion were observed in either CRF or healthy controls. Urinary protein excretion was undetectable for 1 CRF subject and all healthy controls. In the remaining 7 CRF patients, with measurements uncorrected for changes in GFR, BQ-788 produced a small increase in urinary protein excretion ($P<0.01$ versus placebo), but no changes were observed after BQ-123 or BQ-123/788 (Figure 4). After correction for GFR, BQ-788 and BQ-123/788 did not affect proteinuria, but BQ-123 reduced protein leak by 46% ($-8.1\pm4.9$ μg/min; ANOVA $P<0.01$ versus placebo, $P<0.05$ versus BQ-123/788), an effect most apparent in subjects with higher baseline urinary protein excretion.

**Plasma Hormone and BQ-123 Concentrations**

Plasma ET-1 increased to a similar extent after high dose BQ-788 and BQ-123/788 but was unaltered by placebo or BQ-123 (Figure 5). Other hormones were unaffected by endothelin receptor antagonism. BQ-123 was detectable in plasma at 15 minutes after low-dose BQ-123 and at 15 and 45 minutes after high-dose BQ-123 and did not differ between CRF and healthy controls (CRF 1.52±0.36 pg/mL, healthy controls 1.21±0.17 pg/mL at 15 minutes after high dose). BQ-123 concentrations were unaffected by coadministration of BQ-788 (CRF 1.62±0.20 pg/mL, healthy controls 1.26±0.20 pg/mL).

**Discussion**

This is the first clinical study to directly compare ETA, ETB, and combined ETA and ETB receptor antagonism at systemic doses in humans. We have shown in CRF patients that selective ETA receptor antagonism produces substantial re-
ductions in blood pressure associated with renal vasodilation. Additionally, the reduction in filtration fraction and proteinuria in CRF patients suggests a potentially renoprotective action. Combined ETA/B receptor blockade was less effective in lowering blood pressure, had no effect on renal hemodynamics, and reduced ET-1 clearance. Selective ETB receptor antagonism alone produced substantial systemic and renal vasoconstriction. By contrast, in healthy subjects, the systemic hemodynamic effects of ETA and ETA/B receptor blockade were similar to each other but less than those seen in CRF, and there were no effects on renal hemodynamics.

These data confirm the physiological importance of ET-1, through activation of the ETA receptor, in the maintenance of basal systemic but not renal vascular resistance.\(^8,22\) They also show that ET-1 plays a major role in regulating blood pressure and renal vascular resistance in CRF, consistent with activation of the endothelin system in this condition. The reduction in filtration fraction after ETA receptor antagonism suggests an effect primarily on efferent arteriolar tone, which may serve to reduce glomerular pressure. Consistent with our observations, an ETA receptor-selective antagonist has been shown to reduce proteinuria in type 1 diabetes.\(^{23}\)

The effects of BQ-788, whether in the presence of BQ-123 or not, suggest that the net effect of ETB receptor activation on the circulation in health and renal disease is to produce vasodilation. Therefore, similar to observations in healthy subjects\(^{15,24}\) and patients with heart failure,\(^{25}\) any enhanced effects of constrictor ETB receptors that potentially exist in CRF are outweighed by ETB receptor–mediated vasodilation. Of particular interest, ETB receptor antagonism increased renal vascular resistance twice as much as systemic vascular resistance, which suggests that tonic ETB receptor–mediated renal vasodilation plays a key role in maintenance of renal vascular tone. This is likely to be of particular importance in CRF, in which baseline renal vascular resistance is high, and suggests ETA receptor antagonism and not dual ETA/B
blockade might be the best approach in renal patients. However, based on earlier work, the present study was designed to achieve effective and selective blockade of the ETA and ETB receptor and not specifically to reproduce the effects of existing endothelin receptor antagonists, all of which block ETA to a greater extent than ETB receptors. Therefore, although the main effects of therapeutic interest in the present study appear to derive from ETA receptor blockade and are countered by ETB blockade, drugs that cause only modest ETB receptor inhibition might produce similar effects.

Perhaps surprisingly, given the evidence for ETB receptor-mediated natriuresis shown in animal studies, no changes in sodium excretion or fractional excretion were observed in the present study. However, during ETA receptor blockade, despite substantial systemic and renal vasodilation, sodium retention did not occur, which is important if these drugs are to be prescribed safely to patients with CRF.

As a limitation, the present studies were acute studies. We also studied a relatively homogeneous CRF population. Therefore, longer-term studies are now needed in a broader population of patients with kidney disease, including those with low renal perfusion pressure. In particular, because ACE inhibitors are most effective as renoprotective agents in patients with proteinuria >3 g per 24 hours, studies are needed in patients with higher degrees of urinary protein leak to address what may be a major benefit of these agents. Importantly, ethical considerations dictated that patients con-
tinue their regular medications. We therefore cannot exclude an effect of prior drug therapy on our findings. However, patients with CRF generally require several drugs to control blood pressure, and we have been able to demonstrate an effect of endothelin antagonists on top of treatment that included ACE inhibitors in 6 of the 8 patients. Blood pressure and the kinetics of BQ-123 were not significantly different in CRF patients and so are unlikely to account for any differences in renal responses to endothelin antagonists between patients and controls.

In conclusion, we have shown that selective ETA receptor antagonists may be valuable antihypertensive drugs in patients with CRF as well as offering additional benefits, including renoprotection. Endothelin receptor antagonists also improve endothelial function, reduce inflammation and fibrosis, and reverse vascular remodeling and so may offer additional benefits to renal patients, who are at high cardiovascular risk. On this basis, longer-term studies in patients with CRF are justified, with particular attention to effects on proteinuria as a surrogate marker for the progression of renal disease.

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References
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