Blood Pressure–Independent Reduction in Proteinuria and Arterial Stiffness After Acute Endothelin-A Receptor Antagonism in Chronic Kidney Disease

Neeraj Dhaun, Iain M. MacIntyre, Vanessa Melville, Pajaree Lilitkarntakul, Neil R. Johnston, Jane Goddard, David J. Webb

Abstract—Endothelin 1 is implicated in the development and progression of chronic kidney disease and associated cardiovascular disease. We, therefore, studied the effects of selective endothelin-A receptor antagonism with BQ-123 on key independent surrogate markers of cardiovascular risk (blood pressure, proteinuria and renal hemodynamics, arterial stiffness, and endothelial function) in patients with nondiabetic chronic kidney disease. In a double-blind, randomized crossover study, 22 subjects with proteinuric chronic kidney disease received, on 2 separate occasions, placebo or BQ-123. Ten of these subjects also received nifedipine (10 mg) as an active control for the antihypertensive effect of BQ-123. Blood pressure, pulse wave velocity, flow-mediated dilation, renal blood flow, and glomerular filtration rate were monitored after drug dosing. BQ-123 reduced blood pressure (mean arterial pressure: \(-7\pm1\%\); \(P<0.001\) versus placebo) and increased renal blood flow (17\(+4\%\); \(P<0.01\) versus placebo). Glomerular filtration rate remained unchanged. Proteinuria (\(-26\pm4\%\); \(P<0.01\) versus placebo) and pulse wave velocity (\(-5\pm1\%\); \(P<0.001\) versus placebo) fell after BQ-123, but flow-mediated dilation did not change. Nifedipine matched the blood pressure and renal blood flow changes seen with BQ-123. Nevertheless, BQ-123 reduced proteinuria (\(-38\pm3\%\) versus \(26\pm1\%\); \(P<0.001\)) and pulse wave velocity (\(-9\pm1\%\) versus \(-5\pm1\%\); \(P<0.001\)) to a greater extent than nifedipine. Selective endothelin-A receptor antagonism reduced blood pressure, proteinuria, and arterial stiffness on top of standard treatment in renal patients. Furthermore, these studies suggest that the reduction in proteinuria and arterial stiffness is partly independent of blood pressure. If maintained longer term, selective endothelin-A receptor antagonist may confer cardiovascular and renal benefits in patients with chronic kidney disease. (Hypertension. 2009;54:113-119.)

Key Words: endothelin ■ blood pressure ■ arterial stiffness ■ proteinuria ■ chronic kidney disease

Chronic kidney disease (CKD) is common, affecting 6% to 11% of the population in the developed world.\(^1\) Hypertension is a frequent finding in patients with CKD,\(^2\) and its prevalence increases as CKD progresses.\(^3\) Despite treatment with multiple antihypertensive agents, the majority of CKD patients fail to reach target blood pressure (BP).\(^4\) Proteinuria is a common feature of CKD and is independently associated with an adverse renal outcome.\(^5\) Current treatments for proteinuria focus on BP reduction,\(^6\) ideally using angiotensin-converting enzyme (ACE) inhibitors\(^7\) and angiotensin receptor blockers,\(^8\) both of which are thought to reduce proteinuria to a greater extent than accounted for by BP-lowering alone.\(^9\) Nevertheless, many CKD patients have significant residual proteinuria despite optimal treatment.\(^9\)

CKD is also strongly associated with incident cardiovascular disease (CVD).\(^10\) Hypertension\(^10\) and proteinuria\(^11\) make an important contribution to CVD risk in CKD, as do arterial stiffness\(^12\) and endothelial dysfunction.\(^13\) Thus, there remains an unmet need for newer treatments in CKD that will not only lower BP and proteinuria beyond the levels achieved with standard therapies but will also have favorable effects on arterial stiffness and endothelial dysfunction and so offer longer term cardiovascular and renal protection.

Endothelin (ET) 1 is the most potent endogenous vasoconstrictor produced within the vasculature. It is implicated in both the development and progression of CKD.\(^14\) The effects of ET-1 are mediated via 2 receptors, the ETA and ETB receptors, with the major pathological effects in CKD being ETA receptor mediated.\(^14\) However, there are currently few human studies.\(^15,16\) ET-1 also contributes to arterial stiffness\(^17\) and endothelial dysfunction\(^18\) in patients with CVD; however, there are no similar studies in CKD patients.

Our group has shown previously that selective ETA receptor antagonism, but not mixed ETA/B antagonism, reduces BP, increases renal blood flow, and reduces the effective filtration fraction in patients with CKD.\(^15\) However, this was a small
study (n=8) where only 2 of the subjects had overt proteinuria (>300 mg/d), and only 6 were treated with an ACE inhibitor. Thus, based on this work, we hypothesized that, in patients with nondiabetic proteinuric CKD, selective ETA receptor antagonism would reduce proteinuria and arterial stiffness and improve endothelial dysfunction and that these effects would be greater than those achieved with BP reduction alone. Because we have previously demonstrated a synergism between ACE inhibitors and selective ETA receptor antagonism in health, we anticipated that these effects might be evident on top of standard treatment with ACE inhibitors and/or angiotensin receptor blockers.

Methods

Subjects
This was a randomized, double-blind, placebo-controlled study (please also see the online data supplement available at http://hyper.ahajournals.org). Because previous studies with BQ-123 have shown a reduction in BP in CKD patients, and because BP reduction may contribute to changes in arterial stiffness, protein excretion, and natriuresis, nifedipine was used as an open-label active control in a substudy. All of the subjects attended for 2 visits, receiving placebo and BQ-123 in a randomized order, with those taking part in the substudy (nifedipine, 10 mg) randomly chosen and attending for 3 randomized visits. Because previous studies with the same dose of BQ-123 have demonstrated that hemodynamic changes return to baseline after 4 hours, and the half-life of nifedipine is ~2 hours, each visit was separated by ≥7 days to ensure complete washout of the study drugs.

Twenty-two patients with stable proteinuric CKD were recruited into the studies, which were performed between May 2006 and December 2007 in the University of Edinburgh 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.

Drugs
BQ-123 (Clinalfa AG), a selective ETA receptor antagonist, was infused at 1000 nmol/min for 15 minutes. This dose was selected from previous studies as being ETA selective based on plasma BQ-123 concentrations and relating these to the binding constant for the ETA receptor, as well as by a lack of rise in plasma ET-1 concentration after its infusion. The drug was dissolved in physiological saline (15 mL of 0.9%, Baxter Healthcare Ltd) and infused IV at a constant rate of 1 mL/min. Saline vehicle was administered as a placebo.

Nifedipine (10 mg; Adalat, Bayer) was used as an active control and administered orally at the same time that BQ-123 or saline was started in its respective phase. Our choice of active control agent was based, most importantly, on the need for the drug to match the antihypertensive profile of BQ-123, to produce a similar change in renal hemodynamics, and to be a clinically tolerable agent that is also a standard treatment in CKD patients. Although we investigated other agents, nifedipine was the only drug to fulfill all of these criteria.

Para-aminohippurate sodium (PAH; Clinalfa) and inutest (Frese
nius Pharma) were dissolved in 5% dextrose (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, along with a maintenance infusion of 6.6 g/L of PAH and 10 g/L of inutest at a rate of 2 mL/min. For subjects with a calculated glomerular filtration rate (GFR) <50 mL/min or <30 mL/min, doses of PAH and inutest were reduced by one third and two thirds, respectively.

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

Hematocrit was measured on whole blood using a Coulter counter. All of the 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 fructose21 and PAH by high-performance liquid chromatography. Plasma and urine sodium concentrations were measured by using an ion selective electrode and urine protein by using a colorimetric method with pyrogallol red. After extraction, ET-1 was determined by radiommunoassay.

Assessment of Arterial Stiffness and Endothelial Function
Pulse wave velocity (PWV) was used as a measure of arterial stiffness, using the SphygmoCor system (SphygmoCor Mx version 6.3.1, AtCor Medical), in which a high-fidelity micromanometer (SPC-301, Millar Instruments) was used to determine carotid-femoral PWV. Flow-mediated dilation (FMD) was used to assess endothelium-dependent vasomotor function. We did not use glycercyl trinitrate as a measure of endothelium-independent vasomotor function to avoid interference with responses to study drugs. FMD was quantified both as the peak change from baseline and as the area under the curve of the change from baseline in brachial artery diameter after 5 minutes of forearm ischemia.

Study Protocol
On each study day, a cannula was inserted into an antecubital vein in each arm (please also see the data supplement). Diuresis was induced by administering 500 mL of 5% dextrose IV over 30 minutes through the left arm cannula. Thereafter, maintenance infusions of PAH and inutest and 5% dextrose at 180 mL/h continued throughout the study. After a 2-hour equilibration period, baseline measurements were made over 1 hour. Drug or placebo was then administered through the right antecubital cannula or with nifedipine given orally, followed by 4 hours of further measurement. BP, cardiac output (as measured by cardiac index [CI]), and heart rate were recorded throughout the study by well-validated noninvasive automated techniques27-28 every 15 minutes, and urine was collected every 60 minutes by spontaneous voiding.

At the midpoint of each collection period, blood was sampled from the right antecubital cannula for PAH, inulin, sodium, and hematocrit. At 0, 30, 60, 120, and 240 minutes, additional samples were taken for the measurement of plasma ET-1. PWV and FMD were measured immediately before drug and again at 2 and 4 hours after dosing.

Data Analysis
Data were stored and analyzed in Microsoft Excel (version 11.3.7, Microsoft Ltd). BP at each time point was calculated as the mean of 2 recordings and was represented by systolic BP, diastolic BP, and mean arterial pressure (MAP; diastolic BP+1/3 pulse pressure). Bioimpedance data at each time point were calculated as the mean of 4 recordings, each the average of 15 consecutive heartbeats. Data were corrected for body surface area to give CI, for direct comparison between subjects. Systemic vascular resistance index (SVRI) was calculated by dividing MAP by CI and was expressed in dynes per second per meter squared per centimeter². GFR and effective renal plasma flow were calculated from inulin and PAH clearances, respectively. Effective renal blood flow (ERBF) was calculated by dividing the effective renal plasma flow by (1−hematocrit) and effective renal vascular resistance (ERVR) by dividing MAP by ERBF. Urinary protein and sodium excretion (UNaV) were calculated as (urinary protein×urinary flow rate) and (urinary sodium×urinary flow rate), respectively.
study end (Figure 1A and 1B). BQ-123 led to a reduction in versus nifedipine, and BQ-123 versus nifedipine. Responses were of interest were preidentified as placebo versus BQ-123, placebo analysis was performed on untransformed data. Three comparisons corrected change from baseline for substudy results. Statistical SEM change from baseline for drug and placebo and placebo-mean before drug dosing. Hemodynamic and urine results are expressed as For urine data, only 1 baseline measurement was used immediately points that immediately preceded administration of the study drug. Hemodynamic and urine results are expressed as mean±SEM change from baseline for drug and placebo and placebo-corrected change from baseline for study results. Statistical analysis was performed on untransformed data. Three comparisons of interest were preidentified as placebo versus BQ-123, placebo versus nifedipine, and BQ-123 versus nifedipine. Responses were examined by repeated-measures ANOVA, and Bonferroni’s correction was used to assess significance at specific time points. Statistical significance was taken at the 5% level.

Results

All 22 of the CKD patients completed the placebo and BQ-123 phases of the study without adverse events. All of the subjects had similar baseline protein leaks on each study day. Patient diagnoses were IgA nephropathy (n=9), membranous glomerulopathy (n=5), and focal segmental glomerulosclerosis (n=8). For individual subject characteristics and overall demographic data, please see Tables S1 and S2. Subject baseline parameters are shown in the Table.

Main Study

Systemic Hemodynamics

Placebo was associated with increases in systolic BP (127.6±3.3 versus 136.6±3.4 mm Hg; P<0.001), diastolic BP (75.9±1.7 versus 79.9±2.2 mm Hg; P<0.001), MAP (93.1±2.1 versus 98.8±2.4 mm Hg; P<0.001), and SVRI (3360±230 versus 3670±290 dyne/s per m² per cm⁵; P<0.05) from baseline to study end (Figure 1A and 1B). BQ-123 led to a reduction in systolic BP (−14.2±3.0 mm Hg; P<0.001 versus placebo), diastolic BP (−6.8±0.8 mm Hg; P<0.001 versus placebo), MAP (−9.2±1.2 mm Hg; P<0.001 versus placebo), and SVRI (−610±100 dyne/s per m² per cm⁵; P<0.001 versus placebo), with the peak effects at 75 minutes after drug administration. BQ-123 also increased CI (0.3±0.1 L/min per m²; P<0.05 versus placebo). There were no significant differences in the heart rate between placebo and BQ-123 throughout the time course of the study.

Arterial Stiffness and Endothelial Function

Although PWV increased after placebo (7.5±0.4 versus 7.8±0.4 m/s; P<0.001), BQ-123 was associated with a significant fall in PWV (−0.8±0.1 m/s; P<0.001 versus placebo; Figure 1C). With regard to endothelial function, there were no differences in brachial artery FMD response between BQ-123 (4.4±0.5% versus 5.5±0.8%; P=0.056) and placebo (4.4±0.6% versus 5.1±0.7%; P=0.082).

Renal Hemodynamics

Administration of placebo was associated with a gradual reduction in ERBF (1810±233 versus 1454±181 mL/min; P<0.001) and an increase in ERVR (11.5±4.4 versus 12.8±3.8 mm Hg/min per mL; P<0.05) to the study end (Figure 2A and 2B). In contrast, BQ-123 produced a striking increase in ERBF (365±104 mL/min; P<0.01 versus placebo) and a reduction in ERVR (−3.0±0.9 mm Hg/min per mL; P<0.01 versus placebo). There were no significant difference.

### Table. Baseline Data for Main Study and Substudy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Main Study</th>
<th>Substudy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mm Hg</td>
<td>128±3 (99 to 167)</td>
<td>128±3 (108 to 142)</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>75±2 (63 to 95)</td>
<td>76±1 (67 to 84)</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>92±2 (80 to 103)</td>
<td>93±2 (84 to 101)</td>
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<tr>
<td>SVRI, dyne/s per m² per cm⁵</td>
<td>3380±230 (1800 to 5510)</td>
<td>3530±230 (1980 to 5510)</td>
</tr>
<tr>
<td>Cl, L/min per m²</td>
<td>3.0±0.2 (1.8 to 4.7)</td>
<td>2.9±0.2 (1.8 to 4.4)</td>
</tr>
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<td>Heart rate, bpm</td>
<td>56±2 (38 to 75)</td>
<td>57±2 (42 to 66)</td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>7.5±4.0 (5.5 to 12.2)</td>
<td>7.4±0.5 (5.7 to 10.5)</td>
</tr>
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<td>FMD, %</td>
<td>4.4±0.6 (0.6 to 12.7)</td>
<td>4.4±0.9 (0.1 to 8.2)</td>
</tr>
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<td>ERBF, mL/min</td>
<td>1810±233 (106 to 4632)</td>
<td>1968±390 (530 to 4632)</td>
</tr>
<tr>
<td>ERVR, mm Hg/min/L per m²</td>
<td>11.5±4.4 (2.0 to 107.8)</td>
<td>7.1±1.5 (2.0 to 18.2)</td>
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<td>GFR, mL/min per 1.73 m²</td>
<td>43±5 (12 to 99)</td>
<td>43±7 (15 to 99)</td>
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<tr>
<td>UNaV, μEq/min</td>
<td>197±21 (27 to 392)</td>
<td>193±28 (95 to 392)</td>
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<tr>
<td>Urinary protein excretion, μg/min</td>
<td>1570±371 (165 to 8616)</td>
<td>1520±577 (109 to 8616)</td>
</tr>
<tr>
<td>Plasma ET-1 pg/mL</td>
<td>5.7±0.3 (3.6 to 10.5)</td>
<td>6.7±0.5 (3.8 to 8.7)</td>
</tr>
</tbody>
</table>

Values are given as means of baseline pretreatment periods over the 2 or 3 study days±SEM (range).
Maximum equated to throughout the time course of the study. This reduction at its excretion, BQ-123 led to a sustained reduction in proteinuria change in potassium excretion.

Figure 2. Renal hemodynamics and UNaV after ETα receptor antagonism. RBF indicates renal blood flow; RVR, renal vascular resistance; UNaV, sodium clearance. Legend as for Figure 1.

changes in GFRs with either placebo or BQ-123 (maximal change from baseline: 2% for placebo and 3% for BQ-123).

Urinary Sodium and Protein Excretion

Placebo had little effect on UNaV, whereas BQ-123 produced a marked natriuresis with a maximum increase of 36±15 μmol/min (P<0.05 versus placebo; Figures 2C and 3). There was no change in potassium excretion.

Although placebo had little effect on urinary protein excretion, BQ-123 led to a sustained reduction in proteinuria throughout the time course of the study. This reduction at its maximum equated to −496±141 μg/min (P<0.01 versus placebo), a reduction in protein leak of ≈30%. The size of this effect related to baseline urinary protein excretion, with subjects with higher baseline proteinuria achieving a greater reduction (r²=0.78; P<0.05). This effect was seen across all levels of GFR.

Figure 3. Protein excretion after ETα receptor antagonism. Values are given as mean percentage of change from baseline±SEM (left), and mean area under curve (AUC) of the percentage of change from baseline±SEM (right). Black line/block, placebo; gray line/block, BQ-123. +P<0.05 vs placebo, †P<0.01 vs placebo, ‡P<0.001 vs placebo (ANOVA plus Bonferroni correction for significance at specific time points). B. Effect of baseline protein excretion on maximal proteinuria reduction (milligrams per minute) with BQ-123 (r²=0.78; P<0.05). This remains significant (r²=0.29; P<0.05) when the 2 outliers are removed.

Plasma ET-1

There were no changes in plasma ET-1 concentrations with either drug or placebo.

Substudy: Nifedipine (10 mg)

Ten of the 22 subjects took part in a fully randomized 3-way crossover substudy, which included placebo, BQ-123, and nifedipine. Subject baseline parameters are shown in Table 1. All 10 of the subjects completed the 3 phases of the substudy without adverse events.

BQ-123 and nifedipine produced a similar reduction in MAP (please see Figure S1). Nifedipine caused an initial increase in heart rate (mean increase of 10 bpm from baseline) that returned to baseline by 60 minutes. Despite the consistent change in MAP, BQ-123 reduced arterial stiffness to a greater extent than did nifedipine (PWV: −0.6±0.0 versus −0.3±0.1 m/s; P<0.001; please see Figure S1). BQ-123 and nifedipine also increased ERBF to a similar degree (please see Figure S1) and produced a similar natriuresis (at maximum, BQ-123: 64±37 μmol/min; nifedipine: 37±34 μmol/min). By contrast to BQ-123, nifedipine was associated with a gradual increase in urinary protein excretion (at maximum: 190±142 μg/min; P<0.01 versus placebo; please see Figure S1), whereas BQ123 produced a consistent reduction in proteinuria.

Discussion

We have demonstrated that selective ETα receptor antagonism reduces BP, proteinuria, and arterial stiffness in patients with varying degrees of proteinuric nephropathy and that these effects are seen on top of maximally tolerated treatment with ACE inhibitors and angiotensin receptor blockers. Importantly, the reductions in proteinuria and arterial stiffness are greater than those found with an alternative method of BP reduction. These findings suggest a potential role for ETα receptor antagonism in conferring longer-term cardiovascular and renal benefits in patients with CKD.

The current study confirms the importance of ET-1, acting through the ETα receptor, in maintaining the increased vascular tone seen in CKD. There are only 2 studies of the longer-term antihypertensive effects of ET receptor antagonism. These suggest that both selective ETα antagonists and mixed ETαβ antagonists are effective at reducing BP, but neither
study included patients with CKD. Furthermore, both studied untreated hypertensive patients. Our current data suggest that, at least in patients with CKD, where BP control is often difficult, ET receptor antagonism may provide a novel strategy to lower BP to a greater extent than that achieved with existing treatments.

BQ-123 also significantly reduced PWV compared with placebo. This is likely to be attributed largely to the reduction in BP seen with BQ-123. However, PWV continued to fall at the end of the study, even when BP had returned to baseline. Furthermore, when the antihypertensive effect of BQ-123 was matched with nifedipine, the reduction in arterial stiffness was significantly greater with BQ-123. Although nifedipine did cause an expected increase in heart rate, which may have a minor impact on PWV, this had returned to baseline within an hour of the study start and before the measurement of PWV. These observations suggest that the effects of ET<sub>A</sub> receptor antagonism on arterial stiffness seen here are not accounted for by changes in BP alone and should be confirmed in longer-term studies with a range of BP-lowering agents.

There are a few clinical trials demonstrating that differential lowering of PWV with medical treatment results in different cardiovascular or renal outcomes, but the importance of such studies is underscored by epidemiological data suggesting that PWV is an independent risk factor for CVD morbidity and mortality. Karalliedde et al recently showed a BP-independent reduction in PWV with valsartan compared with amlodipine in patients with type 2 diabetes mellitus and proteinuria. Our current data suggest that ET<sub>A</sub> receptor antagonism may reduce arterial stiffness even further in patients already established on blockers of the renin-angiotensin system and, similarly, in a BP-independent manner. However, the effects of ET<sub>A</sub> receptor antagonism on endothelial function, as assessed by FMD, were less impressive, although there was a trend toward improvement. It may well be that these effects take longer to develop.

ET<sub>A</sub> receptor antagonism increased renal blood flow in association with a reduction in renal vascular resistance, suggesting that ET-1, acting through ET<sub>A</sub> receptors, is involved in the increased renovascular tone seen in CKD. We observed no significant changes in GFR, but we did, as in previous studies, see a fall in the filtration fraction (−7% at maximum; data not shown), suggesting that ET-1 induces an ET<sub>A</sub> receptor–mediated vasoconstriction, preferentially affecting the efferent arterioles, although not excluding an effect on mesangial cells and the filtration coefficient. Consistent with a reduction in filtration fraction, BQ-123 produced a sustained reduction in urinary protein excretion that was only beginning to slow at the end of the study. As with the reduction in arterial stiffness, the reduction in proteinuria continued even when the antihypertensive effect of BQ-123 had waned and BP had returned to baseline, suggesting a BP-independent effect. Our control drug, nifedipine, closely matched both the decrease in BP and the increase in renal blood flow seen with BQ-123. Nifedipine acts predominantly at the afferent arteriole, and, so, as expected, it produced steady increases in both GFR (7 mL/min at maximum; data not shown) and proteinuria throughout the study period.

Because BQ-123 had little effect on GFR and substantially reduced filtration fraction and proteinuria over the same time scale as the increase in renal blood flow, these findings are consistent with an action not just at the afferent arteriole but with potential preferential dilatation at the efferent arteriole, similar to, and on top of, that seen with ACE inhibitors.

Although the acute effects on proteinuria described here are likely to be largely explained by systemic and renal hemodynamic changes, it is important to consider other longer-term targets for selective ET<sub>A</sub> receptor antagonism, e.g., the podocyte, which has been implicated in the development of proteinuria. Indeed, a recent study by Wenzel et al showed a reduction in macroalbuminuria in subjects with diabetic nephropathy after 12-week dosing with the ET receptor antagonist avosentan. Interestingly, the authors observed no change in BP, supporting a BP-independent mechanism for the proteinuria reduction seen.

In the current study, the reduction in proteinuria was related to baseline proteinuria, with subjects with a higher level of baseline proteinuria achieving greater reductions. This effect was seen across the range of GFRs. This is similar to the effects seen with ACE inhibitors. Proteinuria reduction is important both for reducing risk of CKD progression and for consequent CVD. Despite maximum achievable renin-angiotensin system blockade, many patients with proteinuric CKD have significant residual proteinuria. Importantly, in this study, all of the subjects were established on treatment with ACE inhibitors, with the majority also taking angiotensin receptor blockers. The ET and renin-angiotensin systems are known to interact, and a synergistic effect, in terms of systemic hemodynamics, has been demonstrated between ET<sub>A</sub> receptor antagonism and both ACE inhibition and angiotensin II type 1 receptor antagonism in humans. Our data suggest that ET<sub>A</sub> receptor antagonism can produce a further reduction in proteinuria of ∼30% on top of that achieved with optimal treatment with inhibitors of the renin-angiotensin system. If maintained longer term, this should reduce both CKD progression and CVD morbidity and mortality.

BQ-123 produced a significant natriuresis. This is likely to be mainly attributable to the increase in renal blood flow seen with ET<sub>A</sub> receptor antagonism. Indeed, nifedipine, which caused a similar change in renal hemodynamics, also caused natriuresis. In addition, all of the subjects showed a net diuresis, even with placebo. These are important observations if ET receptor antagonists are to be used in trials involving CKD patients, in whom salt and water retention is an issue. As a limitation, ours is an acute study, and it will be important to confirm that these effects are maintained longer term, as they are in patients with essential hypertension and pulmonary arterial hypertension. Indeed, the acute studies in these areas predicted the beneficial chronic effects. In addition, we studied a relatively homogeneous CKD population, and further work is needed in a broader population of patients with CKD, including those with diabetes mellitus, vasculitis, and renal vascular disease. Finally, the crossover study design may affect data interpretation and is not ideal for longer-term studies in a larger cohort of subjects.
Perspectives
The current data support a role for selective ET<sub>A</sub> receptor antagonism as a novel and worthwhile therapeutic target in CKD to lower BP, arterial stiffness, and proteinuria on top of traditional treatment, and on this basis, larger and longer-term studies in both diabetic and nondiabetic CKD are justified. Furthermore, with the availability now of both selective ET<sub>A</sub> and mixed ET<sub>A/B</sub> receptor antagonists, a comparison of their renal effects would be of great interest. Indeed, whereas in pulmonary arterial hypertension the studies to date suggest both selective ET<sub>A</sub> antagonism and mixed ET<sub>A/B</sub> antagonism to be of benefit, the current data support selective ET<sub>A</sub> receptor antagonism over mixed blockade in CKD. There have been no major studies as yet in subjects on maximal renin-angiotensin system blockade, across a wide range of GFRs, or looking at natriuresis. Additional studies taking these clinically relevant factors into consideration are now needed. Furthermore, the effects of longer-term treatment on renal hemodynamics remain unclear.

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References


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Running title: Endothelin antagonism & CKD
Supplementary methods

Subjects

Patients with significant co-morbidity, including diabetes mellitus, heart or lung disease, and peripheral vascular disease were excluded. All patients were treated with ACE inhibitors and/or ARBs for their proteinuria. Explicitly, doses of one or both of drugs were titrated to the maximum tolerated, dependent on BP, renal function, serum potassium levels and side effects. All medications were unchanged over the 3 months preceding the studies. 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.

Study protocol

For the duration of the study, subjects were asked to adhere to their usual salt intake. 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 to 24°C with the subject recumbent throughout, except when voiding urine. All patients completed a 24-hour urine collection for quantification of proteinuria on each study day to assess equivalent baseline urinary protein leak between study phases. Patients continued taking their normal medications up to and including each study day with the exception of diuretics, which they omitted that morning.
Supplemental table / figure legends

Table S1: Individual subject characteristics. *To convert to µmol/l, multiply by 88.4. Doses are total per day. IgAN: immunoglobulin A nephropathy; FSGS: focal & segmental glomerulosclerosis; Membranous: membranous glomerulopathy; EPO: erythropoietin.

Table S2: Subject demographic data for main and sub-study. Values are mean ± SEM (range). *To convert to µmol/l, multiply by 88.4. †To convert to mmol/l, multiply by 0.0259.

Figure S1: Systemic and renal hemodynamics, arterial stiffness and protein excretion after ET₄ receptor antagonism and nifedipine 10mg. MAP: mean arterial pressure; RBF: renal blood flow; PWV: pulse wave velocity. Values are given as mean placebo-corrected % change from baseline ± SEM (left) and mean area under curve of placebo-corrected % change from baseline ± SEM (right). Green line/block, nifedipine; red line/block, BQ-123. +p < 0.05 vs. nifedipine, †p < 0.01 vs. nifedipine, *p < 0.001 vs. nifedipine (ANOVA plus Bonferroni correction for significance at specific time points).
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<th>Subject</th>
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<th>Proteinuria (g/d)</th>
<th>BP</th>
<th>ACE inhibitor</th>
<th>ARB</th>
<th>Other drugs</th>
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*To convert to μmol/l, multiply by 88.4.*
Table S2: Subject demographic data for main and sub-study

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<td>28 ± 1 (20 – 37)</td>
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<td>SBP, mmHg</td>
<td>128 ± 3 (107 – 158)</td>
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<td>DBP, mmHg</td>
<td>75 ± 2 (63 – 95)</td>
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<td>MAP, mmHg</td>
<td>93 ± 2 (75 - 118)</td>
<td>93 ± 2 (84 – 101)</td>
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<td>Creatinine(^\ast), mg/dl</td>
<td>2.16 ± 0.33 (0.88 – 5.51)</td>
<td>2.26 ± 1.00 (1.13 – 3.88)</td>
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<td>Cholesterol(^\dagger), mg/dl</td>
<td>170 ± 8 (127 – 228)</td>
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<td>Urinary sodium excretion, mEq/24h</td>
<td>163 ± 18 (39 – 300)</td>
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<td>Urinary protein excretion, g/24h</td>
<td>2.7 ± 0.7 (0.4 - 8.7)</td>
<td>2.9 ± 2.8 (0.4 – 8.0)</td>
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\(^\ast\)To convert to µmol/l, multiply by 88.4. \(^\dagger\)To convert to mmol/l, multiply by 0.0259.
Figure S1. Systemic and renal hemodynamics, arterial stiffness and protein excretion after ET\(_A\) receptor antagonism and nifedipine 10mg