Fractional urinary excretion of endothelin-1 is reduced by acute ETB receptor blockade

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Goddard J, Johnston NR, Cumming AD, Webb DJ. Fractional urinary excretion of endothelin-1 (ET-1) is reduced by acute ETB receptor blockade. Am J Physiol Renal Physiol 293: F1433–F1438, 2007. First published September 12, 2007; doi:10.1152/ajprenal.00101.2007.—Evidence suggests that urinary excretion of endothelin-1 (ET-1) reflects renal ET-1 production and is independent of systemic ET-1 activity. The influence of ET receptors on urinary ET-1 excretion has not been studied in humans, yet peritubular ET receptors are abundant within the kidney. We have studied the effects of acute ETA and ETB receptor blockade with BQ-123 and BQ-788, respectively, on urinary ET-1 excretion in a randomized, placebo-controlled, double-blind study in 16 subjects with a wide range of glomerular filtration rates (15–152 ml/min). Plasma ET-1 concentrations (pET-1) and urinary ET-1 excretion rate (uET-1) at baseline correlated inversely with glomerular filtration rate (GFR) (R² = 0.18 and 0.36, respectively, P < 0.01). However, changes in pET-1 after ET receptor antagonism were not related to changes in uET-1 (R² = 0.007, P = 0.18). pET-1 increased only after BQ-788, alone or in combination with BQ-123, consistent with ETB receptor-mediated clearance of ET-1 from the circulation. uET-1 was reduced only after BQ-788 alone [−4.7 pg/min (SD 5.5), P < 0.01]. Because BQ-788 also reduced GFR, fractional excretion of ET-1 (FeET-1) was calculated. FeET-1 fell after BQ-788 alone [−41% (SD 26%), P < 0.01] or in combination with BQ-123 [−40% (SD 29%), P < 0.01]. FeET-1 was not altered by placebo or BQ-123 alone. In conclusion, urinary ET-1 excretion does not appear to relate to the pool of plasma ET-1. Because of the short duration of this study, it is unlikely that ET receptor blockade had significant effects on renal ET-1 production. Therefore, the reduction in FeET-1 after ETB blockade appears to indicate that renal excretion of ET-1 is at least partly facilitated by ETB receptor activation.

ENDOTHELIN-1 (ET-1) is largely removed from the circulation by receptor (ETB)-mediated mechanisms, primarily in the pulmonary circulation (2, 4, 5). However, the kidneys remove ~10% of ET-1 from the circulation in humans (5). As a small peptide, ET-1 in plasma should be filtered at the glomerulus, and, because clearance from plasma is dependent on filtration, is receptor independent. After filtration, in common with most polypeptides, ET-1 is likely to be hydrolyzed at the brush border and the constituent peptides and amino acids reabsorbed in the tubules and metabolized. In rats, although up to 15% of plasma ET-1 is extracted across the renal bed (2), <1% of injected radiolabeled ET-1 is recovered in the urine (1a). In these isotope studies, the failure of plasma ET-1 to appear in the urine implies that neither glomerular filtration nor tubular secretion of plasma ET-1 accounts for urinary ET-1, which is therefore assumed to be primarily of renal origin. In support of this, cortical interstitial concentrations of ET-1 have been shown to correlate with urinary ET-1 excretion (23).

In chronic kidney disease (CKD), the consequence of a reduced glomerular filtration rate (GFR) is a reduced filtered load of ET-1 delivered to the tubules. Thus renal ET-1 clearance will fall as renal function is lost, contributing to increases in plasma concentrations of ET-1 (11, 16). Renal ET-1 production, and hence urinary ET-1 excretion, is, however, also increased in CKD (1a, 14, 22). Therefore, while plasma ET-1 concentrations and urinary ET-1 excretion may be independent of one other (17), both are related inversely with GFR and may thus appear to be linked. There is also evidence to suggest that ETB receptors may participate in the movement of proteins across the renal tubular epithelium (12) and that peritubular ETB receptors are abundant in the human kidney (10). However, their role in urinary ET-1 excretion has not been studied.

To clarify the dynamic relationship between plasma and urinary ET-1 concentrations, we have studied the effect of changing plasma ET-1 concentrations on urinary ET-1 concentration by acute systemic blockade of ETA and ETB receptors in 16 subjects with GFRs ranging from 15 to 152 ml/min. Serial measurements of plasma ET-1 concentrations and urinary ET-1 excretion were made during a previously reported study (7) of the effects of endothelin receptor antagonism on systemic and renal hemodynamics in patients with renal failure and healthy controls. By measuring the effect of ET receptor blockade on plasma ET-1 concentration and urinary ET-1 excretion, we aimed to establish whether ET receptors play a role in the urinary excretion of ET-1.

METHODS

Subjects. Eight male patients with stable CKD stage 2–5 according to K/DOQI guidelines (1) and eight healthy subjects without CKD matched for age, blood pressure, cholesterol, and weight (Table 1) were recruited to the study, which was 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 sodium. All subjects abstained from alcohol, nicotine, and caffeine-containing products for 24 h and had a light breakfast before attending on each study day. All studies were carried out in a quiet, temperature-controlled room at 22–24°C, with the subject recumbent throughout, except when voiding urine.

Drugs. BQ-123 (Clinalfa, Laufelsingen, Switzerland), a selective ETA receptor antagonist (8), was infused at 100 and 1,000 nmol/min for 15 min at each dose. These doses were selected from a previous

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study as having a threshold and maximum hemodynamic effect in healthy controls (19). BQ-788 (Clinalfa), a selective ETB receptor antagonist (9), was infused at 30 and 300 nmol/min for 15 min, doses shown to be hemodynamically active in a previous systemic dose-ranging study (18). Drugs were dissolved in physiological saline (0.9%; Baxter Healthcare, Thetford, UK) and infused intravenously at a constant rate of 1 ml/min. Saline was administered as placebo. Inutest (Fresenius Pharma) was dissolved in 5% dextrose (Baxter Healthcare) and administered as a bolus loading dose of 3.5 g Inutest in 100 ml dextrose over 15 min, followed by a maintenance infusion of 10 g/l throughout the study at a rate of 2 ml/min. For subjects with a calculated GFR \( < 40 \) ml/min, doses of Inutest were reduced by one-third. All drugs were prepared from sterile stock solutions on the day of the study.

Assays. At prespecified time points, venous blood was collected into EDTA tubes (Sarstedt) for measurement of inulin and plasma ET-1. Additionally, 20-ml aliquots of urine from each voiding were collected into plain tubes for the measurement of urinary inulin and tubes containing 2.5 ml of 50% acetic acid for the measurement of urinary ET-1.

All blood samples were centrifuged immediately at 1,000 \( g \) at \( 4^\circ C \) for 20 min, and plasma and urine were stored in plain tubes at \(-80^\circ C\). Inulin was determined by spectrophotometry after hydrolysis to fructose. After extraction with acetic acid using Bond Elut\textsuperscript{AE} columns (Varian, Harbor City, CA) (15), plasma and urinary ET-1 concentrations were determined by standard radioimmunoassay (Peninsula Laboratories Europe, St. Helens, UK), as previously described (18).

Study protocol. This was a randomized, double-blind, placebo-controlled study originally designed to address the effects of ET antagonism on systemic and renal hemodynamics (7). Subjects attended for four visits, separated by \( \approx 7 \) days, receiving placebo, BQ-123, BQ-788, or a combination of BQ-123 and BQ-788. On each study day, an 18 standard wire gauge (SWG) cannula was sited in an antecubital vein in each arm. Diuresis was induced by 500 ml 5% dextrose over 30 min through the left arm cannula. After 15 min, the loading doses of PAH and Inutest were administered through the same cannula. Thereafter, maintenance infusions of inutest at 120 ml/h and 5% dextrose at 260 ml/h continued throughout the study. Urine was collected every 30 min by spontaneous voiding. Two hours were allowed for inutest and water equilibration, after which baseline

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**Fig. 1.** Plasma endothelin-1 (ET-1) concentrations and urinary ET-1 excretion rates before drug administration (baseline measurements, \( n = 16 \) subjects, 4 visits each). Baseline measurements are the average of 2 plasma samples taken 30 min apart and 2 urine collections of 30-min duration. Baseline measurements were taken after water and inulin loading and equilibration (2.5 h). A: plasma ET-1 concentration vs. glomerular filtration rate (GFR). B: urinary ET-1 excretion rate vs. GFR. C: plasma ET-1 concentration vs. urinary ET-1 excretion rate. D: fractional excretion of ET-1 vs. GFR. (\( \checkmark \)) Healthy subjects; (\( \times \)) chronic kidney disease (CKD) patients.

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## Table 1. Subject demographic data

<table>
<thead>
<tr>
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<th>Healthy Volunteers</th>
<th>Renal Patients</th>
<th>( t )-Test</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>47 (SD 14) (23–64)</td>
<td>46 (SD 13) (25–67)</td>
<td>( P = 0.83 )</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>26 (SD 5) (18–31)</td>
<td>27 (SD 3) (24–33)</td>
<td>( P = 0.63 )</td>
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<tr>
<td>Mean arterial pressure, mmHg</td>
<td>93 (SD 9) (83–104)</td>
<td>99 (SD 10) (79–110)</td>
<td>( P = 0.22 )</td>
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<tr>
<td>Cholesterol, mmol/l</td>
<td>205 (SD 31) (151–236)</td>
<td>217 (SD 23) (174–259)</td>
<td>( P = 0.40 )</td>
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<tr>
<td>Creatinine, mg/dl</td>
<td>0.96 (SD 0.16) (0.70–1.26)</td>
<td>2.89 (SD 1.29) (1.38–4.91)</td>
<td>( P = 0.01 )</td>
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<tr>
<td>GFR, ml/min</td>
<td>102 (SD 24) (65–152)</td>
<td>35 (SD 17) (15–66)</td>
<td>( P &lt; 0.01 )</td>
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<td>Plasma ET-1, pg/ml</td>
<td>4.2 (SD 1.2) (2.9–5.3)</td>
<td>5.6 (SD 1.7) (3.1–10.0)</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>Urine ET-1 excretion, pg/min</td>
<td>8.7 (SD 3.0) (5.1–16.7)</td>
<td>21.3 (SD 9.6) (8.9–40.0)</td>
<td>( P &lt; 0.01 )</td>
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<tr>
<td>FeET-1, %</td>
<td>3.5 (SD 2.0) (1.0–8.7)</td>
<td>15.2 (SD 11.7) (3.2–47.7)</td>
<td>( P &lt; 0.01 )</td>
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Values are given as means (SD) with the range of values given in parentheses. ET-1, endothelin-1; GFR, glomerular filtration rate; FeET-1, fractional excretion of ET-1. To convert cholesterol values to \( \mu \)mol/l, multiply by 88.4. To convert creatinine values to \( \mu \)mol/l, multiply by 0.026.
measurements were made over two 30-min urine collection periods. The low dose of antagonist was then administered through the right antecubital cannula, followed by three 30-min collection periods. The higher dose of antagonist was then administered, followed by five further 30-min collection periods.

At the midpoint of each urine collection period, blood was sampled from the right antecubital cannula for the measurement of inulin. At 0, 60, and 90 min after the start of both low- and high-dose antagonist administration and at the end of the study, further blood and urine samples were taken for the measurement of ET-1 concentrations.

Data analysis. Data were stored and analyzed using a Microsoft Excel data analysis package (Excel 5.0, Microsoft, Wokingham, UK). GFR was calculated from inulin clearance (urinary inulin divided by plasma inulin × urine flow rate). Urinary ET data were expressed as a urinary excretion rate. Fractional excretion of ET-1 was calculated as (plasma ET-1/urine ET-1) divided by (urine inulin/plasma inulin). Relationships between ET-1 and GFR were assessed by linear regression. Responses at set time points after ET receptor antagonism were examined by repeated measures ANOVA. Statistical significance was taken at the 5% level.

RESULTS

Before drug administration, both plasma ET-1 concentration (Fig. 1A) and urinary ET-1 excretion (Fig. 1B) correlated inversely with baseline GFR (plasma ET-1: \( R^2 = 0.18, P < 0.01 \); urinary ET-1: \( R^2 = 0.30, P < 0.01 \)). Additionally, a limited correlation between plasma ET-1 and urinary ET-1 excretion rate (\( R^2 = 0.09, P = 0.02 \)) but not fractional excretion was observed (Fig. 1C). Fractional excretion of endothelin-1 also correlated inversely with baseline GFR (log fractional excretion of ET-1 vs. GFR: \( R^2 = 0.74, P < 0.01 \)) (Fig. 1D).

Urinary ET-1 excretion rate correlated with urinary flow rate for both healthy subjects (\( R^2 = 0.16, P < 0.01 \)) and those with CKD (\( R^2 = 0.08, P < 0.01 \)), but a clear division between these two subject groups was observed, with a greater change in urinary ET-1 excretion per unit change in urine flow rate in CKD patients (Fig. 2).

Plasma ET-1 increased at 30 min after administration of the high dose of BQ-788 alone [change from baseline, +2.6 pg/ml (SD 2.1), \( P < 0.01 \)] or in combination with BQ-123 [+3.5 pg/ml (SD 2.6), \( P < 0.01 \)], but not after placebo [+0.5 pg/ml (SD 1.6), \( P = 0.57 \)] or BQ-123 alone [+0.3 pg/ml (SD 1.3), \( P = 0.90 \)] (Fig. 3). These alterations in plasma ET-1 after ET receptor antagonism were not reflected in changes in urinary ET-1 excretion (\( R^2 = 0.008, P = 0.16 \)) (Fig. 4).

Following administration of the higher dose of BQ-788 alone, urinary ET-1 excretion decreased [−4.7 pg/min (SD 5.5) at 120 min, \( P = 0.01 \)] (Fig. 3), whereas it was not altered by placebo [+0.0 pg/min (SD 4.9), \( P = 0.99 \)], BQ-123 [−0.7 pg/min (SD 5.6), \( P = 0.92 \)] or BQ-123/788 [−0.4 pg/min (SD 5.4), \( P = 0.76 \)].

Because GFR and UFR were also altered by ETB receptor antagonism [GFR: −11% (SD 14%), \( P = 0.00 \) vs. placebo, UFR: −33% (SD 23%), \( P = 0.13 \) vs. placebo], fractional urinary excretion of ET-1 was calculated. This was reduced 30 min after BQ-788 alone [−41% (SD 26%), \( P = 0.00 \)] or in combination with BQ-123 [−40% (SD 29%), \( P = 0.02 \)] but not by placebo [+2% (SD 48%), \( P = 0.75 \)] or BQ-123 alone [−2% (SD 37%), \( P = 0.62 \)] (Fig. 3).

No relationship was found between urinary albumin excretion and urinary endothelin-1 excretion.

DISCUSSION

In this study, in subjects with GFRs ranging between 15 and 152 ml/min, we have demonstrated that plasma ET-1 increases in a linear fashion while urine ET-1 excretion increases exponentially as GFR declines. We have also confirmed, by dynamically altering plasma ET-1 concentrations, the independence of changes in urinary ET-1 excretion from those in plasma ET-1. Our observations are consistent with two independently acting endothelin systems in the systemic circulation and kidney. Additionally, we have demonstrated for the first time that urinary ET-1 excretion is reduced by ETB receptor blockade independent of changes in GFR. Our data suggest a possible role for activation of ETB receptors in the excretion of ET-1 of renal origin, consistent with previous data suggesting the participation of ETB in the movement of proteins across the renal tubular epithelium (12).

While it is known that patients on dialysis or with advanced CKD have higher plasma concentrations and urinary ET-1 excretion rates than normal controls (11, 16), we believe this is the first study to examine the relationship of plasma and urine
Fig. 3. Plasma ET-1 concentration (A), urinary ET-1 excretion rate (B), and fractional excretion of ET-1 (C) after ET receptor blockade. Open bars, placebo; right-hatched bars, BQ-123; left-hatched bars, BQ-123/788; striped bars, BQ-788. Low-dose BQ-123, BQ-788, a combination, or placebo were administered over 15 min after baseline measurements (t = 0 min). High-dose BQ-123, BQ-788, a combination, or placebo were administered over 15 min at t = 90 min. Plasma was sampled for ET-1 concentration at t = 0, 30, 90, 120, 180, and 240 min. Urine was collected every 30 min throughout the study, and urine ET-1 concentration was measured at the same time points as plasma ET-1. *p < 0.05 vs. baseline.
ET-1 to GFR across all CKD stages. Our baseline data, before pharmacological maneuvers to change plasma ET-1, confirm an inverse linear correlation between plasma ET-1 concentration and GFR, consistent with reduced renal filtration of ET-1 and thus renal removal from the circulation, and an inverse exponential relationship between urine ET-1 excretion and GFR, consistent with increased renal production of ET-1 in CKD. Because both plasma ET-1 concentrations and urine ET-1 excretion are related to GFR, a weak correlation exists between these two measurements at baseline. However, we have demonstrated in this study that increases in plasma ET-1 concentrations, consequent on blockade of the ETB receptor (3) alone, or in combination with ETA receptor blockade, are not reflected in changes in urinary ET-1 excretion and GFR, consistent with increased renal production of ET-1 in CKD. Because both plasma ET-1 concentrations and urine ET-1 excretion are related to GFR, a weak correlation exists between these two measurements at baseline. However, we have demonstrated in this study that increases in plasma ET-1 concentrations, consequent on blockade of the ETB receptor (3) alone, or in combination with ETA receptor blockade, are not reflected in changes in urinary ET-1 excretion and GFR, consistent with increased renal production of ET-1 in CKD. Additionally, our baseline data demonstrate that fractional excretion of ET-1 increases exponentially as GFR falls. Animal models have shown, using isotope studies (1a), that plasma ET-1 does not account for urinary ET-1 and that renal cortical interstitial ET-1 levels determined by microdialysis in normal and remnant rat kidneys in vivo correlate directly with urine ET-1 excretion (23), supporting urinary ET-1 excretion as being a marker of the increased renal ET-1 production in CKD. Our findings of an exponential increase in urine ET-1 excretion as CKD progresses are in keeping with this.

ET-1 is produced by most renal cells, but there are few data, as yet, to suggest how renal ET-1 reaches the tubules. Proximal tubular ET-1, produced in vitro in response to exposure to protein, is largely secreted basolaterally into the interstitium (26). Whether interstitial endothelin is actively secreted or moves into the tubular lumen as a consequence of passive diffusion is not known. Urine ET-1 excretion has previously been shown to increase with increasing urine flow rate (24, 25), a finding confirmed in this study. An increase in urine flow rate will reduce luminal accumulation of solutes and so increase the concentration gradient between the interstitium and the tubular lumen, increasing the movement of diffusible compounds down this concentration gradient. The relationship of urine ET-1 excretion to urine flow rate could, therefore, be potentially explained by passive diffusion of renal ET-1 into the tubules. We also observed that the increase in urine ET-1 excretion for any given increase in urine flow rate was significantly greater in patients with CKD. If ET-1 does move into the tubular lumen by passive diffusion, then the increased renal levels of ET-1 in CKD (1a) would result in a greater concentration gradient between tubular cells and lumen than in healthy controls, and hence, again, greater excretion of urine ET-1 for any given urine flow rate. Changes in urine flow rate, however, do not account fully for the reduction in fractional excretion of ET-1 seen after ETB receptor blockade in this study, as urine flow rate was not significantly altered by dual receptor blockade with BQ-123 and BQ-788 but a reduction in fractional excretion of ET-1 was still observed after both ETB and combined ETA/B receptor blockade. Similarly, alterations in renal hemodynamics cannot fully explain this reduction in fractional excretion of ET-1 as, while BQ-788 resulted in a fall in GFR and renal blood flow, dual ETA/B antagonism did not alter renal hemodynamics.

In vitro studies suggest that exposure of tubules to luminal protein has effects on tubular reabsorptive function (6). In this study, seven of eight patients with CKD had measurable proteinuria. However, we could not find any relationship between urinary albumin excretion and urinary ET-1 excretion to explain the relationships between GFR and ET-1 excretion in this study. Additionally, the changes in urinary ET-1 excretion and fractional excretion after ETB receptor blockade were seen equally in the CKD patients and the healthy subjects (who had no proteinuria). We do not think, therefore, that our results are explained by concurrent urinary protein excretion.

A study in teleost fish has localized ETB receptors to the basolateral aspect of proximal tubular cells and has demonstrated that low concentrations of ET-1 are able to reduce cell-to-tubular lumen transport of methotrexate and cyclosporin derivatives. This effect was inhibited by ETB receptor antagonists (12) and appears to be nitric oxide dependent (13), suggesting a role for ET-1 acting via ETB receptors in tubular transport mechanisms. Additionally, ETB receptor-deficient rats demonstrate an attenuated increase in urinary excretion of ET-1 on a high-salt diet compared with wild-type rats, suggesting a role for ETB receptors specifically in the excretion of renal ET-1 (21). In our study, after a correction for changes in GFR by calculating fractional excretion of ET-1, it was evident that urinary ET-1 excretion is reduced specifically by blockade of the ETB receptor either alone or when combined with ETA receptor blockade.

It is possible that ET-B blockade could be reducing ET-1 production within the kidney. Suga et al. (20) have demonstrated that this is possible over a time course of 4–8 days in a rat model. The change in fractional excretion of ET-1 in our study was seen 30 min after high-dose ETB blockade and persists for a further 60 min but appears to return to baseline by the end of the study. Changes in urinary ET-1 in this study are acute and transitory and we think are unlikely, therefore, to be due to changes in ET-1 production. Thus we propose that the
renal tubular excretion of ET-1 is at least partly mediated through activation of the ETB receptor.

In conclusion, our data provide confirmatory evidence in humans for at least two independent endothelin systems, one in the circulation and one in the kidney, both related to GFR. Our observations suggest, for the first time, that urinary excretion of ET-1 may be facilitated, in part, by tubular ETB receptor activation. The clinical consequences of ETB receptor blockade causing a reduction in urinary ET-1 excretion are not known.

REFERENCES


