Haemodynamic and renal effects of endothelin receptor antagonism in patients with chronic kidney disease

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Abstract

Background. Endothelin-1 (ET-1) has been implicated in the pathophysiology of chronic kidney disease (CKD) and ET receptor blockade has shown renoprotective effects in animals. We examined the haemodynamic and renal effects of an ET receptor antagonist, TAK-044, in patients with CKD.

Methods. Seven patients with CKD (mean arterial pressure 103 mmHg; mean plasma creatinine 3.5 mg/dl) received three 15 min intravenous infusions, each separated by at least 7 days, of either placebo or TAK-044 (100 or 750 mg) in a randomized, double blind crossover study. Systemic and renal haemodynamics, and plasma immunoreactive ET-1, big ET-1 and C-terminal fragment concentrations, were determined before and after the infusions of placebo and drugs.

Results. Compared with placebo, TAK-044 reduced mean arterial pressure (MAP) (100 mg: 7.4 ± 1.9 mmHg, 750 mg: 8.4 ± 2.3 mmHg, P < 0.01) and systemic vascular resistance index (100 mg: 650 ± 140 dyne.s.cm⁻².m⁻², 750 mg: 829 ± 141 dyne.s.cm⁻².m⁻², P < 0.01) at both doses. TAK-044 increased cardiac index and heart rate to a similar degree at both doses. With regards to renal haemodynamics, TAK-044 had no significant effect on the glomerular filtration rate at either dose but tended to increase renal plasma flow (100 mg: 9.6 ± 5.0 ml/min, 750 mg: 25.3 ± 19.5 ml/min) and decreased the effective filtration fraction (100 mg: 3.6 ± 1.1%, 750 mg: 4.7 ± 1.7%, P < 0.01), in a dose-dependent manner. TAK-044 had no significant effect on sodium or lithium clearance, or on fractional excretion of sodium and lithium. Plasma ET-1 concentrations rose more than two-fold after 750 mg TAK-044 while big ET-1 and C-terminal fragment concentrations were unchanged.

Conclusions. These findings suggest an important role for ET-1 in controlling systemic and renal haemodynamics in patients with CKD. The antihypertensive and potentially renoprotective actions of ET receptor antagonists shown in this study may prove useful in slowing the progression of CKD. Clinical trials are now needed to address these key questions for CKD.

Keywords: chronic kidney disease; endothelin antagonism

Introduction

The endothelin (ET) family of peptides are potent vasoconstrictor and vasopressor agents [1]. ET-1 is the principal vascular isoform, which interacts with two receptor subtypes, ET_A and ET_B [2,3]. In human resistance vessels, ET_A and ET_B receptors are found on vascular smooth muscle cells, where they mediate vasoconstriction [4]. The ET_B receptor is also found on vascular endothelial cells where it mediates vasodilatation by the release of nitric oxide and prostacyclin [4]. In addition, the ET_B receptor has a role in ET-1 clearance from the circulation [5]. ET receptors are widely distributed within the human kidney, with the ET_A subtype localized to vascular smooth muscle, notably in the glomeruli, vasa recta and arcuate arteries, whereas ET_B receptors are more numerous (ET_B to ET_A ratio 2:1) and more widespread, with a high concentration in the collecting system [6,7]. With respect to the renal system, ET-1 has a role in the paracrine/autocrine regulation of renal and intrarenal blood flow, glomerular haemodynamics and sodium and water homeostasis [8].

Plasma concentrations of ET-1 are substantially raised in chronic kidney disease (CKD) [9], likely reflecting a balance between increased vascular...
production and decreased renal clearance of ET-1. These concentrations may contribute to the increased vascular tone and high incidence of cardiovascular mortality associated with CKD, as well as play a role in its progression [8]. Renal ET-1 production is also increased in CKD, again suggesting a role for renal ET-1 in its progression [10]. In support of this hypothesis, ET receptor antagonists have been shown to increase renal blood flow and slow the rate of progression of renal failure in animal models [11,12]. In healthy subjects, systemic blockade of ETA receptors alone or both ETA and ETB receptors lowers blood pressure (BP) and systemic vascular resistance (SVR) [13–16] and increases effective renal plasma flow (ERPF) [16–18]. Recently, we have demonstrated that, in patients with CKD, selective blockade of ETA receptors alone or both ETA and ETB receptors reduces BP and systemic vascular tone. Selective ETA receptor antagonism, but not non-selective ETA/B receptor blockade, also increases renal blood flow and reduces renal vascular resistance in CKD patients, suggesting a role for the ETA receptor in the increased renal vascular tone seen in CKD [16]. Selective ETA receptor antagonism also reduced the effective filtration fraction (EFF) and proteinuria [16], suggesting a favourable and potentially renoprotective, effect on efferent arteriolar tone and intra-glomerular pressure.

In this study, we examined the actions of brief administration of a ‘non-selective’ ETA/B receptor antagonist, TAK-044, on systemic and renal haemodynamics, as well as renal tubular function, in patients with CKD. We also measured the effect of brief ET receptor antagonism on the plasma concentrations of the precursor big ET-1 and the two products of cleavage by the ET converting enzyme, the mature ET-1 peptide and the inactive C-terminal fragment (CTF).

Methods

Patients

The study was prospectively powered to show significant systemic haemodynamic effect on the primary endpoint of mean arterial pressure (MAP). Seven men with stable, dialysis-independent CKD (age 45±3 years) participated in this study, which was conducted with the approval of the local ethics review committee and with the written informed consent of each volunteer. Subjects were relatively homogeneous in terms of diagnoses, degree of renal impairment and number and type of medications they were prescribed compared with the range of diagnoses and drugs seen within a standard CKD population. Subjects had plasma creatinine concentrations >1.7 mg/dl and no change in renal function in the 2 months preceding the study. All subjects abstained from alcohol, nicotine and caffeine-containing products for 24 h and had a light breakfast before attending on each study day. They had no other significant comorbidity apart from hypertension. All medications (Table 1) were withheld on the morning of the studies.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>Obstructive nephropathy</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>Polycystic kidneys</td>
<td>Enalapril/furosemide/metoprol</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>IgA nephropathy</td>
<td>Enalapril</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>IgA nephropathy</td>
<td>Doxazosin/enalapril/metoprol</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>Glomerulosclerosis</td>
<td>Doxazosin/amlopidine/</td>
</tr>
<tr>
<td>6</td>
<td>65</td>
<td>Obstructive nephropathy</td>
<td>Bendroflumethazide</td>
</tr>
<tr>
<td>7</td>
<td>46</td>
<td>Hypertensive nephropathy</td>
<td>Furosemide/metoprol/enalapril</td>
</tr>
</tbody>
</table>

Subject 1 completed placebo and 100 mg TAK-044 phases. Subjects 2–7 completed all three study phases. (IgA, Immunoglobulin A).

Drug

TAK-044 is a cyclic hexapeptide ET receptor antagonist. As the selectivity of TAK-044 for the ETA receptor is 100-fold compared with that, for the ETB receptor, it is classified as a ‘non-selective’ ET receptor antagonist [19]. However, TAK-044 is still 18-fold more selective for the ETA than the ETB receptor. This balance of ETA:ETB receptor selectivity is very similar to that of bosentan (20-fold ETA receptor selective), currently the only licensed ET receptor antagonist (for pulmonary arterial hypertension). In this study we used 100 mg of TAK-044 as the lower dose and 750 mg as the higher dose of drug. Based on previous data, 100 mg of TAK-044 blocks >95% of ETA receptors with the degree of ETB receptor blockade being >5 but <75%. At the higher dose of TAK-044, >99% of ETA receptors were blocked and the degree of ETB receptor blockade is >75% [20]. Furthermore, the higher dose of 750 mg of TAK-044 produces a clinically relevant reduction in BP [20].

Assessments

BP was measured using a well-validated semi-automated technique [21] and cardiac function (stroke volume, cardiac output and heart rate) was measured using a non-invasive bioimpedance method [22]. ERPF and glomerular filtration rate (GFR) were estimated by measuring the renal clearances of p-aminohippurate sodium (PAH) and inulin, respectively [23]. Proximal tubular re-absorptive capacity of sodium was assessed using a physiologically inert dose of oral lithium carbonate [24]. Venous blood samples for separate assay of plasma IR ET-1, big ET-1 and CTF concentrations were collected, stored and analysed as previously described [20].

Study design

Subjects participated in a three-phase, double-blind, randomized, placebo-controlled crossover study, with at least 7 days between phases. The detailed protocols for administration of TAK-044 [14] and conducting clearance studies [25] have been previously described. In brief, subjects attended at 7:00 pm on the night before the study and received oral lithium carbonate (Camcolit, 250 mg
sustained release; Norgine, Harefield, UK) 14 h before baseline measurements. On each study day, two intravenous cannulae were placed in the antecubital fossa of the non-dominant arm for infusion of PAH/inulin and TAK-044 (100 or 750 mg given over 15 min), or placebo (dextrose 50 ml). A third cannula was placed in the antecubital fossa of the opposite arm for blood sampling. Priming doses of PAH and inulin were followed by maintenance infusions. After an equilibration period, blood samples were drawn and urine collected at the beginning and at the end of accurately timed 30-min clearance periods. A volume of water (to replace urine and blood losses minus intravenous volume infused) was drunk by the subjects after all urine collections sufficient to maintain a urine output of 10 ml/min throughout the study. Two baseline collection periods preceded and four followed infusion of TAK-044 or placebo.

Data analysis

BP at each time point was calculated as the mean of two recordings and represented as MAP (MAP = diastolic BP + one-third pulse pressure). Bioimpedance data at each time point was calculated as the mean of four recordings, each the average of 15 consecutive heart beats. Systemic vascular resistance index (SVRI) was calculated by dividing MAP by cardiac index (CI) and expressed in dyn.s.cm \(^{-5}\).m\(^{-2}\). GFR and ERPF were calculated from inulin and PAH clearances, respectively. EFF was calculated as \(\frac{\text{GFR} \times \text{ERPF}}{100}\). Urinary sodium excretion was calculated as urinary sodium × urinary flow rate and fractional excretion of sodium (FE\(\text{Na}\)) as (urinary sodium × plasma inulin)/(plasma sodium × urine inulin). This was similar for lithium excretion and fractional excretion of lithium (FELi). Data are presented as mean ± standard error of the mean (SEM).

Data were analysed statistically by repeated measures analysis of variance (ANOVA). Factors included in the ANOVA were subject, dose of TAK-044, time point and dose-time point interaction. In none of the analyses (except plasma ET-1 concentrations, which were analysed as absolute values by the Kruskal–Wallis non-parametric test with Dunn’s multiple comparisons post-tests) was there any evidence of a statistically significant dose-time point interaction. Therefore, the adjusted dose group means from the ANOVA were compared with the null hypothesis by a two-sided Student's t-test. Statistical analyses were performed by the use of the software package SAS (version 6.07, SAS Institute Inc.).

Results

Patient diagnoses and medications are presented in Table 1. Five of the seven patients were hypertensive at screening, with MAP >105 mmHg. Mean baseline measurements of subjects prior to dosing are shown in Table 2. Patient 1 withdrew after completing the TAK-044 100 mg and placebo phases and was, therefore, included in the analysis. All the other subjects completed all three phases.

| Table 1. Five of the seven patients were hypertensive at screening, with MAP >105 mmHg. Mean baseline measurements of subjects prior to dosing are shown in Table 2. Patient 1 withdrew after completing the TAK-044 100 mg and placebo phases and was, therefore, included in the analysis. All the other subjects completed all three phases. |

| Table 2. Mean baseline (mean of the three pre-dose values in each study phase) values before administration of TAK-044 or placebo in subjects with CKD |

<table>
<thead>
<tr>
<th>Subjects</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>57 ± 3</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>103 ± 4</td>
</tr>
<tr>
<td>SVRI (dyn.sec.cm (^{-5}).m(^{-2}))</td>
<td>3649 ± 425</td>
</tr>
<tr>
<td>CI (L.min(^{-1}).m(^{-2}))</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Serum creatinine (mg.dl(^{-1}))</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>GFR (ml.min(^{-1}))</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>ERPF (ml.min(^{-1}))</td>
<td>110 ± 25</td>
</tr>
<tr>
<td>EFF (%)</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>FENa (%)</td>
<td>7.1 ± 0.6</td>
</tr>
<tr>
<td>FELi (%)</td>
<td>26.7 ± 2.7</td>
</tr>
<tr>
<td>ET-1 (pM)</td>
<td>34.5 ± 6.5</td>
</tr>
<tr>
<td>Big ET-1 (pM)</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>CTF (pM)</td>
<td>5.0 ± 0.7</td>
</tr>
</tbody>
</table>

Systemic haemodynamics

TAK-044 significantly lowered MAP by 7.4 ± 1.9 mmHg after 100 mg and 8.4 ± 2.3 mmHg after 750 mg TAK-044 (for both P < 0.01). These decreases in MAP occurred despite significant increases in CI with both doses (Figure 1) and an increase in heart rate after 100 mg TAK-044 (Figure 1). There were, therefore, substantial decreases in SVRI of 650 ± 140 dyn.s.cm \(^{-5}\).m\(^{-2}\) after 100 mg and 829 ± 141 dyn.s.cm \(^{-5}\).m\(^{-2}\) after 750 mg TAK-044 (for both P < 0.01).

Renal haemodynamics

TAK-044 had little effect on GFR at either dose. There was a trend for both doses of TAK-044 to increase ERPF (100 mg: 9.6 ± 5.0 ml/min; 750 mg: 25.3 ± 19.5 ml/min; Figure 2). EFF was decreased by 3.6 ± 1.1% after 100 mg and by 4.7 ± 1.7% after 750 mg TAK-044 (for both P < 0.01; Figure 2).

Renal sodium handling

TAK-044 had no significant effects on sodium or lithium clearance or on the FENa or FELi at either dose (Table 3).

Plasma endothelin peptides

TAK-044 750 mg, but not 100 mg, significantly increased circulating plasma immunoreactive (IR) ET-1 concentrations (~3-fold; Table 4). Increases were maximal at the end of the 15 min TAK-044 infusion and waned rapidly. TAK-044 had no effect on plasma big ET-1 or CTF (data not shown).

TAK-044 pharmacokinetics

Peak serum concentrations of TAK-044 (C\(_{\text{max}}\)) were observed at 15 min (T\(_{\text{max}}\)) following the start of infusion at both 100 mg and 750 mg doses.
At 1 h post-dose TAK-044 concentrations had fallen to <10% of their peak (data not shown).

Discussion

In this study, we have added to the data suggesting that ET-1 contributes to the maintenance of vascular tone in patients with CKD [16,26] and shown for the first time that brief administration of a ‘non-selective’ ET receptor antagonist not only reduces BP but also...
but decreased clearance of the peptide. This is a feature of ETB receptor blockade comes into play. This is analogously to the response to angiotensin-converting enzyme (ACE) inhibitors, where reduced efferent arteriolar tone is associated with a reduction in proteinuria and a slowing of CKD progression [32].

Indeed, the observed haemodynamic changes in our patients with chronic heart failure, or those with pulmonary arterial hypertension, the effects seen in acute studies were sustained for a longer term [19] and, in essential hypertension, prolonged administration of the orally active, non-selective ET\textsubscript{A/B} receptor antagonist, bosentan, resulted in sustained lowering of BP [29]. Importantly, chronic low dose administration of bosentan had no effect on heart rate suggesting the changes seen with TAK-044 in the present study may represent a reflex response to substantial systemic vasodilation [29].

In the present study, TAK-044 tended to increase ERPF. There was little change in GFR. As a consequence of these trends, EFF was reduced modestly after both doses of TAK-044. Again, these results are similar to those in healthy volunteers [28]. Interestingly, there was a trend for the higher dose of TAK-044 to reduce BP and SVRI and increase ERPF and so reduce EFF to a greater degree than the lower dose. Thus, both selective ET\textsubscript{A} and non-selective ET\textsubscript{A/B} receptor antagonism led to an improvement in systemic and renal haemodynamics, but the effects with dual receptor blockade tended to be greater than with ET\textsubscript{A} receptor antagonism alone. These results may, in part, be explained by the effects of ET-1 on the sympathetic nervous system (SNS). Both selective ET\textsubscript{A} and ET\textsubscript{B} receptor blockade have been shown to reduce the potentiating effects of ET-1 on the vasoconstriction seen with mediators of the SNS [30,31].

These data support previous work by our group using selective antagonists to block ET\textsubscript{A} and ET\textsubscript{B} receptors alone and in combination in CKD patients [16] and these changes in renal haemodynamics are suggestive, in the absence of a change in glomerular filtration coefficient, of a preferential action of TAK-044 on the efferent arteriole. This situation is analogous to the response to angiotensin-converting enzyme (ACE) inhibitors, where reduced efferent arteriolar tone is associated with a reduction in proteinuria and a slowing of CKD progression [32].

Table 3. Effects of TAK-044 (100 mg or 750 mg) given intravenously over 15 min on renal sodium handling; mean changes (\(\Delta\)) from basal over 2 h are shown, corrected for changes with placebo

<table>
<thead>
<tr>
<th>Time from drug (min)</th>
<th>Placebo</th>
<th>TAK-044 100 mg</th>
<th>TAK-044 750 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>15</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>60</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>120</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

\(P < 0.05\) for comparison from placebo.

**Table 4.** Effect of intravenous infusion of placebo or TAK-044 100 mg and 750 mg on immunoreactive (IR) plasma endothelin (pM)

<table>
<thead>
<tr>
<th>Time from drug (min)</th>
<th>Placebo</th>
<th>TAK-044 100 mg</th>
<th>TAK-044 750 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.7</td>
<td>4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>15</td>
<td>4.8</td>
<td>4.8</td>
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<tr>
<td>60</td>
<td>4.9</td>
<td>4.9</td>
<td>4.9</td>
</tr>
<tr>
<td>120</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

\(P < 0.05\) for comparison from placebo.
study are very similar, both qualitatively and quantitatively, to those observed in the first 2h after administration of both ACE inhibitors and angiotensin AT₁ receptor antagonists [33,34]. Of note, however, in this study the reduction in EFF seen with TAK-044 occurred even though four of the seven patients studied were already being treated with ACE inhibitors. This would suggest that ET receptor antagonism may be potentially useful in addition to ACE inhibition in the treatment of CKD. Indeed, this combination has been shown to be more renoprotective than either agent alone in a rat model of progressive CKD [35] and recently, in healthy subjects, combined treatment with an ACE inhibitor and an ET receptor antagonist was shown to reduce EFF more than either treatment alone [36].

Our group has previously published data supporting a role for selective ETA over combined ETA/ETB receptor antagonism in patients with CKD [16]. To reconcile these results with those of the current study with the ‘non-selective’ ET receptor antagonist TAK-044, it is important to recognize that previous studies were designed to explore strategies of substantial ETA and ETB receptor blockade alone and in combination. In the current study, the lower dose of TAK-044 selectively antagonized the ETA receptor, with the higher dose providing an additional degree of ETB receptor blockade. Thus, these current data are in line with our earlier work, but suggest that ‘non-selective’ antagonists, like TAK-044, provide effects that are more in line with selective ETA receptor antagonism, with higher doses providing non-selective ETA/B antagonism. The current study, together with previous animal and human data [8], support selective ETA receptor antagonism over non-selective ETA/B blockade as a treatment option in patients with CKD.

Endogenously generated renal ET-1 is thought to promote sodium excretion and inhibit water re-absorption [1], through actions mediated by the ETB receptor. Systemic infusion of ET-1, however, reduces salt and water excretion [37,38], likely secondary to ET-1 mediated renal vasoconstriction. Previous studies addressing selective ETA and combined ETA/B and receptor antagonism do not show any adverse effect on renal sodium excretion in healthy subjects or patients with CKD despite a significant fall in BP [15,16]. It is only when selective ETA receptor antagonism is combined with ACE inhibition that a significant natriuresis is observed, an effect that appears to be dependent on both an unblocked ETB receptor and the bioavailability of nitric oxide [36]. In the current study, TAK-044 had no net effect on renal sodium or lithium handling. However, any potential anti-natriuretic effect of tubular ETB receptor antagonism may have been counteracted by the glomerular haemodynamic changes resulting from vascular ETA receptor antagonism. Furthermore, it is possible that antagonism of the tubular (mainly ETB) actions of ET-1 only occurs with higher renal concentrations of TAK-044. Overall, the actions of TAK-044 on renal sodium handling do not discourage the further development of non-selective ET receptor antagonists for use in CKD.

In conclusion, we have shown that systemic ‘non-selective’ ETA/B receptor blockade with TAK-044 leads to systemic vasodilation and lowers BP, in patients with CKD. In addition, TAK-044 reduces EFF, with only minor changes in GFR and ERPF and no changes in renal sodium handling. The antihypertensive actions of ET receptor antagonists may be useful clinically in treating the hypertension associated with CKD. Furthermore, the potentially anti-atherogenic properties of ET receptor antagonists could be useful in reducing the high cardiovascular morbidity and mortality observed in CKD patients. By having similar actions on renal haemodynamics as ACE inhibitors and potentially adding to the effects of these agents, ET receptor antagonists may also have clinical utility in slowing the progression of CKD. As previous studies suggest the renal benefits of ET receptor antagonism to be related largely to selective ETA receptor blockade, the ability of a so-called ‘non-selective’ ET receptor antagonist to show improvements in renal haemodynamics is encouraging. Dosing of such drugs, in particular with relation to the degree of achieved ETB receptor blockade, may be an important consideration for future clinical studies. We appreciate the relatively small sample size of this study but these results are encouraging for the further development of ET receptor antagonists for the treatment of CKD and longer-term studies, which are already ongoing in diabetic nephropathy, seem warranted by the growing evidence of potential benefit.

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