Selective Endothelin-A Receptor Antagonism Reduces Proteinuria, Blood Pressure, and Arterial Stiffness in Chronic Proteinuric Kidney Disease

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Abstract—Proteinuria is associated with adverse cardiovascular and renal outcomes that are not prevented by current treatments. Endothelin 1 promotes the development and progression of chronic kidney disease and associated cardiovascular disease. We, therefore, studied the effects of selective endothelin-A receptor antagonism in proteinuric chronic kidney disease patients, assessing proteinuria, blood pressure (BP), and arterial stiffness, key independent, surrogate markers of chronic kidney disease progression and cardiovascular disease risk. In a randomized, double-blind, 3-way crossover study, 27 subjects on recommended renoprotective treatment received 6 weeks of placebo, 100 mg once daily of sitaxsentan, and 30 mg once daily of nifedipine long acting. Twenty-four–hour proteinuria, protein:creatinine ratio, 24-hour ambulatory BP, and pulse wave velocity (as a measure of arterial stiffness) were measured at baseline and week 6 of each treatment. In 13 subjects, renal blood flow and glomerular filtration rate were assessed at baseline and week 6 of each period. Compared with placebo, sitaxsentan reduced 24-hour proteinuria (−0.56±0.20 g/d; P=0.0069), protein:creatinine ratio (−38±15 mg/mmol; P=0.0102), BP (−3.4±1.2 mm Hg; P=0.0069), and pulse wave velocity (−0.64±0.24 m/s; P=0.0052). Nifedipine matched the BP and pulse wave velocity reductions seen with sitaxsentan but did not reduce proteinuria. Sitaxsentan alone reduced both glomerular filtration rate and filtration fraction. It caused no clinically significant adverse effects. Endothelin-A receptor antagonism may provide additional cardiovascular and renal protection by reducing proteinuria, BP, and arterial stiffness in optimally treated chronic kidney disease subjects. The antiproteinuric effects of sitaxsentan likely relate to changes in BP and renal hemodynamics. (Hypertension. 2011;57:772-779.)

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

Chronic kidney disease (CKD) is common, affecting 6% to 11% of the population globally.1 It is strongly associated with incident cardiovascular disease (CVD).2 Proteinuria is a common feature of CKD, and the degree of proteinuria is closely associated with renal outcome and cardiovascular events.3 Importantly, a reduction in proteinuria is associated with a slowing of both the decline in glomerular filtration rate (GFR)3 and the progression to end-stage renal disease.3 In addition, proteinuria reduction is associated with an improved cardiovascular outcome in both those with4 and without5 CKD. Thus, a reduction in proteinuria is now widely accepted as a surrogate end point for progression and cardiovascular disease risk. In a randomized, double-blind, 3-way crossover study, 27 subjects on recommended renoprotective treatment received 6 weeks of placebo, 100 mg once daily of sitaxsentan, and 30 mg once daily of nifedipine long acting. Twenty-four–hour proteinuria, protein:creatinine ratio, 24-hour ambulatory BP, and pulse wave velocity (as a measure of arterial stiffness) were measured at baseline and week 6 of each treatment. In 13 subjects, renal blood flow and glomerular filtration rate were assessed at baseline and week 6 of each period. Compared with placebo, sitaxsentan reduced 24-hour proteinuria (−0.56±0.20 g/d; P=0.0069), protein:creatinine ratio (−38±15 mg/mmol; P=0.0102), BP (−3.4±1.2 mm Hg; P=0.0069), and pulse wave velocity (−0.64±0.24 m/s; P=0.0052). Nifedipine matched the BP and pulse wave velocity reductions seen with sitaxsentan but did not reduce proteinuria. Sitaxsentan alone reduced both glomerular filtration rate and filtration fraction. It caused no clinically significant adverse effects. Endothelin-A receptor antagonism may provide additional cardiovascular and renal protection by reducing proteinuria, BP, and arterial stiffness in optimally treated chronic kidney disease subjects. The antiproteinuric effects of sitaxsentan likely relate to changes in BP and renal hemodynamics.

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mediated via 2 receptors, the ET\textsubscript{A} and ET\textsubscript{B} receptors, with the major pathological effects in CKD being ET\textsubscript{A} receptor mediated.\textsuperscript{13} However, there are currently few human studies in CKD.\textsuperscript{14--17} The aim of the current study was to evaluate whether the oral ET\textsubscript{A} receptor antagonist, sitaxsentan, is able to reduce proteinuria, BP, and arterial stiffness longer term in subjects with chronic nondiabetic proteinuric kidney disease.

Methods

Subjects

We enrolled subjects 18 to 70 years of age with stable CKD stages 1 to 4\textsuperscript{18} and proteinuria (>300 mg/d). Subjects were on treatment with ACE inhibitors and/or ARBs (but not necessarily diuretics) 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 adverse effects. All of the medications were unchanged over the 3 months preceding the studies.

Patients with significant comorbidity, including diabetes mellitus, heart or lung disease, and peripheral vascular disease, were excluded. To enhance homogeneity and avoid other influences on vascular reactivity, patients with vasculitis, other systemic inflammatory disease, polycystic kidney disease, and nephrotic syndrome were excluded. Furthermore, we excluded patients with abnormal liver enzymes, hemoglobin <8 g/dL, and women of childbearing potential.

Thirty-three patients with stable proteinuric CKD were screened, and 27 were recruited into the studies. These were performed between June 2007 and March 2009 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.

Study Protocol

This was a single-center, 3-phase randomized, double-blind, placebo-controlled crossover study. Its purpose was to investigate the safety, tolerability, and efficacy of 100 mg of sitaxsentan once daily, placebo, and 30 mg of nifedipine LA once daily versus placebo on reduction of proteinuria (primary end point), the safety, tolerability, and efficacy of 100 mg of sitaxsentan once daily, placebo, and 30 mg of nifedipine LA once daily on renal hemodynamics. This was a single-center, 3-phase randomized, double-blind, placebo-controlled crossover study. Its purpose was to investigate the safety, tolerability, and efficacy of 100 mg of sitaxsentan once daily, placebo, and 30 mg of nifedipine LA once daily on renal hemodynamics.

Subjects were randomly assigned to receive 100 mg of sitaxsentan, matched placebo, or 30 mg of nifedipine LA once daily for 6 weeks, in addition to their usual medications. Each phase was separated by a minimum 2-week washout period. Proteinuria, BP, and arterial stiffness were assessed at baseline, week 3, and week 6 of each treatment period (Figure S1, available in the online Data Supplement at http://hyper.ahajournals.org). Proteinuria was assessed using both the mean 24-hour protein excretion and the mean protein:creatinine ratio of 3 consecutive 24-hour urine collections. Ambulatory BP was recorded at the brachial artery using a validated SpaceLabs 90217 ambulatory BP monitor.\textsuperscript{22} Measurements were taken every 30 minutes for 24 hours, and mean systolic BP, mean arterial pressure (MAP), and diastolic BP were calculated. As measures of arterial stiffness, pulse wave velocity (PWV) and central augmentation index (cAIx) were recorded\textsuperscript{23} using the SphygmoCor system (SphygmoCor Mx, AtCor Medical, version 6.31) and assessed postdose as described fully elsewhere.\textsuperscript{24} Safety data were obtained at baseline and at weeks 1, 2, 3, 4, and 6 for each treatment period. These included “office” BP, weight, hemoglobin, hematocrit, liver enzymes, serum potassium, and adverse effects.

Renal Function Substudy

For those subjects taking part in the substudy, para-aminohippurate sodium (PAH; Clinalfa) and Inutest (Fresenius Pharma) clearances were used to assess renal blood flow and GFR, respectively, at baseline and week 6 of each of the 3 study periods. These followed our standard protocol and are described fully elsewhere.\textsuperscript{14,15}

Plasma ET-1

Plasma ET-1 was measured at baseline, week 3, and week 6 of each treatment period. After extraction,\textsuperscript{25} ET-1 was determined by radioimmunoassay.\textsuperscript{26}

Data and Statistical Analysis

Data were stored and analyzed using SAS version 8.2 or higher. The planned sample size (an approximate target of 30 subjects to be enrolled in the main study) was based primarily on logistical and clinical considerations. However, data from a previous study\textsuperscript{14} using an ET\textsubscript{A} receptor antagonist administered to 22 subjects in a crossover design reported a reduction in proteinuria of up to −496 μg/min with a SE of 141 μg/min. This is ∼0.7 g/dl with an SD of 0.9 g/dl. Using these data, it is possible to show that the current study size would have 80% power to detect such a difference at the 2-sided 5% significance level. Of the 30 subjects to be enrolled in the main study, the aim was for 24 subjects to complete and ∼15 subjects were to be included in the substudy, with the aim of 12 subjects completing that. Similar calculations demonstrate that the substudy had ∼50% power to detect statistically significant changes, but the substudy was exploratory in nature, with the principal aim of examining trends in the data.

For efficacy end points, the changes from baseline to week 6 and from baseline to week 3 were analyzed using a mixed model with repeated measures. The model was implemented using PROC MIXED in SAS with terms for treatment group (fixed effect, categorical variable), baseline value (fixed effect, continuous variable, as appropriate for the end point), period effect (fixed effect, categorical variable), week (the “repeated” effect), week-by-treatment interaction, and subject-by-period interaction (the “subject” blocking effect). The model was fitted using restricted maximum likelihood estimation, and an autoregressive covariance structure was implemented. Least squares means estimates for each treatment and the treatment differences (100 mg of sitaxsentan minus placebo; 100 mg of sitaxsentan minus 30 mg of nifedipine LA, and 30 mg of nifedipine LA minus placebo) were generated for weeks 3 and 6 from the treatment group-by-week interaction. Associated SEMs and P values were calculated. The assumptions of the model were checked by investigation of a normal probability plot of standardized residuals and a plot of standardized residuals versus fitted values. Carryover and sequence effects were explored by adding these terms into the model (and removing if nonsignificant). In addition, all of the data were summarized using simple summary statistics (observed means, medians, and SDs). Percentage changes were summarized for each treatment. Median differences between treatments were calculated, and 95% CIs for the differences were derived using the Hodges-Lehman estimator.

For the renal substudy, GFR and effective renal plasma flow were calculated from inulin and para-aminohippurate sodium clearances, respectively. Effective renal blood flow (ERBF) was calculated by dividing effective renal plasma flow by (1–hematocrit), and effective renal vascular resistance by dividing MAP by ERBF. Effective filtration fraction (EFF) was defined as GFR/ERBF.
Role of the Funding Source
This study was designed by the academic authors. The sponsor was responsible for generating the subject randomization schedule, gathering the data from the investigational site to create the clinical database, and for data unblinding. On the basis of an analysis plan developed in collaboration with the academic authors, who also took responsibility for interpretation of the data and for submitting this article for publication, the sponsor did the data analysis. All of the authors had full access to study results after unblinding the data.

Results
All 27 of the subjects completed all 3 phases of the study. Patient diagnoses were IgA nephropathy (n=14; 52%), focal segmental glomerulosclerosis (n=6; 22%), membranous nephropathy (n=3; 11%), hypertensive nephrosclerosis (n=2; 7%), reflex nephropathy, and microhematuria of presumed glomerular origin (n=1; 4%, for both), and 1 subject had an unknown cause for his or her CKD. Subject baseline parameters are shown in Table 1. For all of the subjects, baseline parameters did not differ among the 3 study phases.

Table 1. Baseline Patient Characteristics for Main Study and Substudy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Main Study (n=27)</th>
<th>Substudy (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>48±12</td>
<td>46±13</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>23 (85)</td>
<td>12 (92)</td>
</tr>
<tr>
<td>White, based on the subjects’ history</td>
<td>27 (100)</td>
<td>13 (100)</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29.3±4.6</td>
<td>28.2±4.7</td>
</tr>
<tr>
<td>Twenty-four–h BP, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>125±12</td>
<td>127±10</td>
</tr>
<tr>
<td>Diastolic</td>
<td>78±7</td>
<td>80±8</td>
</tr>
<tr>
<td>Mean</td>
<td>94±78</td>
<td>95±7</td>
</tr>
<tr>
<td>Creatinine, mg/dL*</td>
<td>1.73±0.85</td>
<td>1.72±0.76</td>
</tr>
<tr>
<td>Estimated GFR, mL/min per 1.73 m²</td>
<td>54±26</td>
<td>55±26</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>136±18</td>
<td>132±16</td>
</tr>
<tr>
<td>Serum potassium, mmol/L</td>
<td>4.6±0.4</td>
<td>4.6±0.4</td>
</tr>
<tr>
<td>Cholesterol, mg/dL†</td>
<td>178±32</td>
<td>168±38</td>
</tr>
<tr>
<td>Urinary protein excretion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/24 h</td>
<td>2.03±1.7</td>
<td>2.01±1.6</td>
</tr>
<tr>
<td>PCR, mg/mmol</td>
<td>156±143</td>
<td>150±144</td>
</tr>
<tr>
<td><strong>Arterial stiffness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PWV, m/s</td>
<td>8.3±2.4</td>
<td>7.4±1.4</td>
</tr>
<tr>
<td>AIx, %</td>
<td>28±12</td>
<td>24±14</td>
</tr>
<tr>
<td><strong>Medications, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>18 (67)</td>
<td>10 (77)</td>
</tr>
<tr>
<td>ARB</td>
<td>11 (41)</td>
<td>3 (23)</td>
</tr>
<tr>
<td>ACE inhibitor + ARB</td>
<td>5 (19)</td>
<td>2 (15)</td>
</tr>
<tr>
<td>No ACE inhibitor or ARB</td>
<td>3 (11)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>α-Blocker</td>
<td>6 (22)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>8 (30)</td>
<td>4 (31)</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>3 (11)</td>
<td>3 (23)</td>
</tr>
<tr>
<td>Diuretic</td>
<td>2 (7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Statin</td>
<td>18 (67)</td>
<td>8 (62)</td>
</tr>
</tbody>
</table>

Values are given as mean of 3 baseline pretreatment periods±SD unless otherwise specified.
*To convert to micromoles per liter, multiply by 88.4.
†To convert to millimoles per liter, multiply by 0.0259.

Figure 1. Effects of placebo, sitaxsentan, and nifedipine LA (30 mg) on the coprimary end points of (A) 24-hours proteinuria, and (B) PCR. Values are given as mean percentage of change from baseline±SEM at week 3 and week 6. Gray block, placebo; hashed block, sitaxsentan; black block, nifedipine. C, Effect of baseline protein excretion on maximal proteinuria reduction (grams per day) with sitaxsentan ($r^2=0.67; P<0.01$).
Sitaxsentan Versus Placebo

Proteinuria

Placebo was associated with no significant changes in 24-hour urinary protein excretion or protein:creatinine ratio (PCR) from baseline to week 3 or week 6. Sitaxsentan, however, significantly reduced both 24-hour proteinuria and PCR by \( \approx 30\% \) by study end (Figure 1). These effects of sitaxsentan on proteinuria were apparent at week 3 of the study period. The observed means (\( \pm SD \)) for 24-hour proteinuria were 2.07 ± 1.77 g/d at baseline and 1.34 ± 1.16 g/d at week 6. For PCR these were 156 ± 147 and 109 ± 109 mg/mmol. For 24-hour proteinuria, the least squares mean changes (\( \pm SEM \)) at week 6 were \(-0.73 \pm 0.14 \) g/d for sitaxsentan and \(0.09 \pm 0.14 \) g/d for placebo (\( P=0.0001 \)). For PCR, these were \(-48 \pm 10 \) and \(8.6 \pm 10 \) mg/mmol (\( P=0.0002 \)). Sitaxsentan reduced proteinuria by \( \approx 25\% \) in 19 (70\%) of 27 subjects and by \( \approx 40\% \) in 9 (33\%) of 27 subjects. Only 2 subjects failed to show a reduction in 24-hour urine protein excretion and only 1 in PCR. Furthermore, the degree of proteinuria reduction closely related to the baseline urinary protein excretion, with subjects with higher baseline proteinuria achieving a greater reduction (\( r^2 = 0.67; P<0.01 \)). This effect was seen across all levels of GFR (data not shown).

BP and Arterial Stiffness

Although placebo did not significantly affect MAP, systolic BP, or diastolic BP between baseline and week 6 of the study period, sitaxsentan reduced all 3 of the parameters by \( \approx 5 \) mm Hg after 3 and 6 weeks dosing (Figure 2).

Placebo had no significant effects on PWV or cAIx over the 6-week study period, whereas sitaxsentan reduced both by study end. PWV fell by \( \approx 5\% \) compared with baseline, a difference of \( \approx 8\% \) compared with placebo (Hodges-Lehman 95\% CI: \(-16\% \) to \(-2\% \); Figure 3).
Sitaxsentan Versus 30 mg of Nifedipine LA

After 6 weeks of dosing there were no significant differences between sitaxsentan and nifedipine in the reductions from baseline in BP parameters. Systolic BP was reduced by \(-4.3 \pm 1.2\) versus \(-4.4 \pm 1.2\) mm Hg, diastolic BP by \(-3.7 \pm 0.8\) versus \(-2.7 \pm 0.8\) mm Hg, and MAP by \(-3.9 \pm 0.9\) versus \(-3.4 \pm 0.9\) mm Hg (least squares mean \(\pm\) SEM for sitaxsentan and nifedipine, respectively). Despite this, sitaxsentan reduced proteinuria to a significantly greater extent than nifedipine (24-hour proteinuria: \(-0.61 \pm 0.14\) versus \(0.0 \pm 0.14\) g/d, \(P=0.0033\); PCR: \(-43 \pm 10\) versus \(-5.6 \pm 10\) mg/mmol, \(P=0.0134\)). For sitaxsentan, the reduction in proteinuria correlated with the fall in MAP at week 6 (\(r^2=0.16; P=0.04\)). However, there were no relationships for the changes in proteinuria and BP for the placebo and nifedipine phases. Although PWV fell to a similar degree with nifedipine as with sitaxsentan (\(-0.4 \pm 0.2\) versus \(-0.4 \pm 0.2\) m/s), only sitaxsentan reduced cAlx after 6 weeks of dosing.

All of the changes in proteinuria, BP, and arterial stiffness had returned to baseline before starting the next phase of the study (minimum 2 weeks). There were no changes in plasma ET-1 concentrations with placebo, sitaxsentan, or nifedipine.

Renal Substudy

ERBF did not change from day 0 to week 6 with placebo, sitaxsentan, or nifedipine. Although GFR was similar at day 0 and week 6 with placebo and nifedipine, sitaxsentan produced a substantial fall in GFR by week 6. EFF remained unchanged between day 0 and week 6 with both placebo and nifedipine. However, EFF was lower with sitaxsentan. This was a consistent finding, with 12 of 13 subjects demonstrating a fall in EFF (\(n=13\); Table 2 and Figure 4). Ten subjects had an EFF of >20% at baseline. These subjects showed a fall of >2% (range: 2.1% to 8.9%) after 6 weeks of sitaxsentan treatment. The 3 subjects with an EFF <20% at baseline showed less impressive reductions in EFF after sitaxsentan dosing. All of the changes in renal hemodynamics had returned to baseline before starting the next phase of the study (minimum 2 weeks).

Adverse Events

There was no difference in the overall incidence of adverse events between the sitaxsentan and placebo groups (Table 3). Of note, there was no significant weight gain (see Figure S1), fall in hemoglobin or hematocrit, or rise in serum potassium associated with sitaxsentan treatment compared with placebo.

Discussion

We have demonstrated that sitaxsentan, an oral selective ETA receptor antagonist, reduces proteinuria, BP, and arterial stiffness in patients with proteinuric nephropathy. These effects were seen in patients already receiving optimal treatment with ACE inhibitors and ARBs and were at least in part BP independent. These findings suggest a potential role for ETA receptor antagonism in conferring longer-term cardiovascular and renal benefits in patients with CKD.

Proteinuria reduction is important both for reducing risk of CKD progression and associated CVD.\(^3,4\) However, despite maximum achievable renin-angiotensin system blockade, many patients with proteinuric CKD have significant residual proteinuria.\(^9\) In the current study all of the subjects were established on maximally tolerated treatment with ACE

Table 2. Renal Substudy Data From Clearance Studies Performed at Baseline and Week 6 of Each Study Period

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo Baseline</th>
<th>Placebo Week 6</th>
<th>Sitaxsentan Baseline</th>
<th>Sitaxsentan Week 6</th>
<th>Nifedipine Baseline</th>
<th>Nifedipine Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR, mL/min</td>
<td>56 ± 7</td>
<td>54 ± 8</td>
<td>57 ± 8</td>
<td>48 ± 8</td>
<td>59 ± 8</td>
<td>58 ± 9</td>
</tr>
<tr>
<td>ERBF, mL/min</td>
<td>533 ± 66</td>
<td>532 ± 65</td>
<td>511 ± 63</td>
<td>543 ± 73</td>
<td>562 ± 82</td>
<td>530 ± 72</td>
</tr>
<tr>
<td>ERVR, mm Hg/min per l</td>
<td>230 ± 52</td>
<td>206 ± 39</td>
<td>236 ± 44</td>
<td>232 ± 48</td>
<td>248 ± 58</td>
<td>254 ± 56</td>
</tr>
<tr>
<td>EFF, %</td>
<td>19.1 ± 1.1</td>
<td>17.9 ± 1.3</td>
<td>20.8 ± 1.0</td>
<td>16.6 ± 0.7</td>
<td>20.3 ± 1.1</td>
<td>20.5 ± 1.4</td>
</tr>
</tbody>
</table>

Values are given as predosing baseline \(\pm\) SEM. GFR indicates glomerular filtration rate; ERBF, effective renal blood flow; ERVR, effective renal vascular resistance; EFF, effective filtration fraction.

Figure 4. Effect of placebo, sitaxsentan, and nifedipine LA (30 mg) on EFF. Individual subject data are presented, as well as the mean \(\pm\) SEM at baseline and 6 weeks.
inhibitors and/or ARBs with good BP control. Despite this, mean baseline proteinuria was still significant at ≈2 g/d (range: 0.3 to 7.8 g/d). Importantly, the data presented here support a potential role for ET receptor antagonists as a novel class of drug to help further reduce proteinuria in these patients on top of standard therapy. This should have the capacity to reduce CKD progression and the associated CVD, morbidity, and mortality.

Interactions between the ET and renin-angiotensin systems are well established. Furthermore, we have shown recently that acute ET<sub>A</sub> receptor antagonism can reduce proteinuria by an additional ≈30% on top of that achieved with optimal treatment with inhibitors of the renin-angiotensin system in subjects with proteinuric CKD. The current study suggests that these effects are maintained longer term and are of a similar magnitude. Interestingly, the size and time course of this effect are similar to those seen with blockers of the renin-angiotensin system. Furthermore, of those subjects showing ≥40% reduction in urinary protein leak (9 of 27), 4 were on dual ACE inhibitor/ARB therapy, supporting a role for ET receptor antagonists as adjunctive treatments for CKD patients already established on renin-angiotensin system inhibitors. As has been shown previously with ACE inhibitors, the reduction in proteinuria was related to baseline proteinuria, with subjects with a higher level of baseline urinary protein leak achieving greater reductions. This effect was seen across the range of renal function studied.

The effects of sitaxsentan on proteinuria described here are likely explained by changes in both systemic and renal hemodynamics. As expected, there was a correlation between the reductions in BP and proteinuria after 6 weeks of sitaxsentan dosing (r² = 0.16; P = 0.04). However, sitaxsentan also reduces proteinuria through BP-independent effects. Other longer-term targets for selective ET<sub>A</sub> receptor antagonism include the podocyte, which has been implicated in the development of proteinuria. In a recent study, the ET receptor antagonist avosentan reduced macroalbuminuria in subjects with diabetic nephropathy in the absence of a change in BP. In the current study, our active control nifedipine matched the fall in BP seen with sitaxsentan, but despite this, sitaxsentan reduced proteinuria to a greater degree. Furthermore, for the reduction in BP seen with sitaxsentan (≈4 mm Hg) a less impressive fall in proteinuria than the observed at ≈30% would be expected. ACE inhibitors that reduce proteinuria by a similar degree to the effect seen here with sitaxsentan have more impressive effects on BP, reducing it by ≈10 mm Hg.

Our substudy data support a renal hemodynamic mechanism for the reduction in proteinuria seen with sitaxsentan. ET<sub>A</sub> receptor antagonism had no effect on renal blood flow or renal vascular resistance. However, as in previous studies, there was a very consistent fall in filtration fraction (−4%), suggesting that ET-1 induces an ET<sub>A</sub> receptor-mediated preferential efferent arteriolar constriction. These effects are analogous to, and occur in addition to, those seen with renin-angiotensin system blockade. This postulated reduction in efferent arteriolar tone with ET<sub>A</sub> receptor antagonism should reduce glomerular perfusion pressure. This will result in a reduction in proteinuria with an associated short-term fall in GFR. Consistent with this proposed effect, we observed a significant fall in GFR (−9 mL/min) after 6 weeks of sitaxsentan treatment. In patients already prescribed blockers of the renin-angiotensin system, these effects, despite an initial fall in GFR, should correlate with longer-term slowing of the rate of CKD progression.

The current study confirms the concept that blocking the ET<sub>A</sub> receptor reduces BP in CKD. Sitaxsentan reduced BP modestly (a fall in MAP of ≈4 mm Hg). This effect may have been more impressive had the subjects not had such good baseline BP control. Previous studies of the longer-term antihypertensive effects of ET receptor antagonism suggest that both selective ET<sub>A</sub> and mixed ET<sub>A/B</sub> antagonists are effective at reducing BP in untreated hypertensive patients or those with resistant hypertension. 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.

Sitaxsentan also significantly improved arterial stiffness as measured by PWV and cAIx compared with placebo. This is likely to be attributable largely to the reduction in BP seen with sitaxsentan. Interestingly, despite similar BP effects, sitaxsentan reduced cAIx to a greater extent than nifedipine. In the current study, unlike for BP and proteinuria, the reductions in PWV and cAIx were higher at 6 weeks than after 3 weeks of sitaxsentan treatment. It is possible that longer treatment with an ET<sub>A</sub> receptor antagonist might reduce PWV further and perhaps to a greater degree than nifedipine. There are 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.

Six weeks of sitaxsentan dosing in subjects with varying degrees of proteinuric CKD was not associated with any more adverse events than placebo. Importantly, we observed no weight gain, clinically significant edema, fall in hemoglobin
or hematocrit, or rise in serum potassium. Furthermore, the changes in renal hemodynamics were not associated with sodium retention (data not shown). Fluid retention has been observed in several trials with ET receptor antagonists, although its mechanism remains unclear. ET-1 acts in the renal tubule via the ET_B receptor to promote natriuresis and diuresis. Thus, edema could be aggravated by mixed ET_A/B antagonists, and its absence in the current study may be explained by the selective ET_A blocking nature of our drug. In addition, the careful selection of our subjects, excluding those with clinically apparent CVD and overt heart failure (and, thus, a propensity to fluid overload), may also help. From a renal perspective, the lack of rise in serum potassium with sitaxsentan is clinically significant, because this is a troublesome adverse effect with both ACE inhibitors and ARBs limiting their use.

Perspectives
We recognize some limitations to the current work. The study was crossover by design. This may lead to subjects dropping out, limiting its power, as well as having the issue of carryover effects between different treatment phases. However, carryover was not a significant factor in the statistical analysis, and the results from the main study clearly indicate that the power was adequate. Subjects were optimized for treatment of their proteinuria with ACE inhibitors and ARBs but were not necessarily prescribed diuretics. These may potentiate the antiproteinuric effects of renin-angiotensin system blockade. Thus, the current data apply only to those subjects not taking diuretics. Furthermore, although the small study number is reasonable to show benefits of treatment, much larger studies are required to highlight potentially important but infrequent adverse events. In summary, the current data support a role for selective ET_A receptor antagonism as a novel and worthwhile therapeutic target in CKD to lower proteinuria, BP, and arterial stiffness on top of standard treatment, and on this basis, larger and longer-term studies are now justified.

Addendum
Sitaxsentan has been voluntarily withdrawn by Pfizer, Ltd due to unacceptable side effects. However, the findings in this manuscript remain true for selective endothelin A receptor antagonism.

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References

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Supplementary material- Figure S1

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Running title: Endothelin antagonism & CKD
Registration number at www.clinicalTrials.gov: NCT00810732
Supplemental Figure 1.

- Regular medications including ACE-I and ARB continued

- Sitaxsentan 100mg, Nifedipine LA 30mg or placebo as per randomization

- Safety data obtained at baseline, week 1, 2, 3, 4 & 6

- Washout

- ≥ 2 weeks