

Endothelin-A Receptor Antagonism Modifies Cardiovascular Risk Factors in CKD

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ABSTRACT

Arterial stiffness and impaired nitric oxide (NO) bioavailability contribute to the high risk for cardiovascular disease in CKD. Both asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO production, and endothelin-1 (ET-1) oppose the actions of NO, suggesting that ET-1 receptor antagonists may have a role in cardiovascular protection in CKD. We conducted a randomized, double-blind, three-way crossover study in 27 patients with proteinuric CKD to compare the effects of the ET_A receptor antagonist sitaxentan, nifedipine, and placebo on proteinuria, BP, arterial stiffness, and various cardiovascular biomarkers. After 6 weeks of treatment, placebo and nifedipine did not affect plasma urate, ADMA, or urine ET-1/creatinine, which reflects renal ET-1 production; in contrast, sitaxentan led to statistically significant reductions in all three of these biomarkers. No treatment affected plasma ET-1. Reductions in proteinuria and BP after sitaxentan treatment was associated with increases in urine ET-1/creatinine, whereas reduction in pulse-wave velocity, a measure of arterial stiffness, was associated with a decrease in ADMA. Taken together, these data suggest that ET_A receptor antagonism may modify risk factors for cardiovascular disease in CKD.

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CKD is common, affecting 6%–11% of the population globally.¹ It is strongly associated with incident cardiovascular disease (CVD).² This increased cardiovascular risk is not adequately explained by conventional (Framingham) risk factors, such as hypertension, hypercholesterolemia, diabetes mellitus, and smoking, all of which are common in patients with CKD. Thus, emerging cardiovascular risk factors have been an area of intense investigation.³ Arterial stiffness⁴ makes an important independent contribution to CVD risk in CKD, and this is promoted by both conventional and emerging cardiovascular risk factors.

Hyperuricemia and a shift in the balance of the vasodilator nitric oxide (NO)

and vasoconstrictor endothelin (ET) systems have been identified as potential contributors to increased cardiovascular risk in patients with CKD.⁵ These are all common in a typical CKD population.^{3,6} Epidemiologic studies report a relationship between serum uric acid and a wide variety of cardiovascular conditions, including hypertension, diabetes mellitus, coronary artery disease, cerebrovascular disease, and CKD.⁷ Indeed, serum uric acid is considered by some to be an independent risk factor for both CVD^{8,9} and CKD.¹⁰ Others have noted that an elevated serum uric acid level predicts the development of hypertension and CKD.⁷ Of note, emerging clinical data show that decreasing serum uric acid

levels has both cardiovascular and renal benefits.^{11–13}

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO synthases. By inhibiting NO formation, ADMA causes endothelial dysfunction, vasoconstriction, elevation of BP, and progression of experimental atherosclerosis.¹⁴ ADMA concentrations are increased in patients with CKD,¹⁴ and clinical data support ADMA as an independent marker of CKD progression, cardiovascular morbidity, and overall mortality.^{15–17} Studies have shown a reduction in ADMA after therapy in patients with hypertension and hypercholesterolemia,^{18,19} but not in patients with CKD.

ET-1 is a potent endogenous vasoconstrictor produced within the vasculature. It is implicated in both the development and progression of CKD.²⁰ Its effects are mediated *via* two receptors, the ET_A and ET_B receptors; the major pathologic effects are ET_A receptor mediated.²⁰ We have recently shown that long-term selective ET_A receptor

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antagonist therapy using the orally active drug sitaxentan reduces proteinuria, BP, and arterial stiffness in patients with proteinuric CKD,²¹ effects that are potentially renoprotective. We hypothesized that in this same cohort of patients with CKD, sitaxentan would also reduce levels of serum uric acid, ADMA, and urine ET-1 (as a measure of renal ET-1 production) and so provide broader cardiovascular and renal protection. The current data show the effects of sitaxentan, as well as placebo and an active control agent, nifedipine, on these novel cardiovascular risk factors.

As described elsewhere,²¹ after 6 weeks of dosing no significant differences were seen between sitaxentan and nifedipine in the reductions from baseline in BP measures. Despite this, sitaxentan reduced proteinuria to a significantly greater extent than did nifedipine. Pulse-wave velocity (PWV)—a measure of arterial stiffness—decreased to a similar degree with nifedipine as with sitaxentan. Placebo did not affect proteinuria, BP, or PWV (see Table 1 for summary data). Thirteen of the 27 patients took part in a renal substudy, which showed that sitaxentan alone reduced both GFR and effective filtration fraction (Table 2). Of note, sitaxentan did not cause increases in patients' weight or hematocrit that would suggest extracellular fluid accumulation.

Baseline serum uric acid was in the frankly hyperuricemic range in all three phases of the study: placebo, 476 ± 20

$\mu\text{mol/L}$; sitaxentan, $506 \pm 21 \mu\text{mol/L}$; nifedipine, $479 \pm 19 \mu\text{mol/L}$. Baseline serum uric acid was inversely related to baseline proteinuria ($r^2=0.19$; $P=0.02$). Whereas placebo and nifedipine had no effect on serum uric acid, sitaxentan reduced serum uric acid by approximately 11% by study end (Figure 1A). This effect was similar at weeks 3 and 6 of the study. In multivariate analysis, the reduction in serum uric acid was not associated with changes in proteinuria, BP, or PWV (data not shown). The reduction in serum uric acid was matched by an increase in the fractional urinary excretion of uric acid (baseline versus week 6: $6.0\% \pm 0.6\%$ versus $7.3\% \pm 0.7\%$; $P=0.05$).

Baseline ADMA concentrations were the same for all three phases of the study: placebo, $0.52 \pm 0.01 \mu\text{mol/L}$; sitaxentan, $0.52 \pm 0.01 \mu\text{mol/L}$; nifedipine, $0.52 \pm 0.02 \mu\text{mol/L}$. Whereas placebo and nifedipine did not affect ADMA, 6 weeks of sitaxentan reduced ADMA by approximately 8% (Figure 1B). This reduction in ADMA was directly correlated with a reduction in PWV (Figure 2A; $r=0.39$; $P<0.05$), and in multivariate analysis, the change in ADMA (but not changes in proteinuria, BP, plasma ET-1, urine ET-1/creatinine, or serum uric acid) independently predicted the reduction in PWV after sitaxentan treatment.

Plasma ET-1 concentrations were similar at baseline in all three phases of the study—placebo, $3.57 \pm 0.50 \text{ pg/ml}$; sitaxentan, $3.60 \pm 0.49 \text{ pg/ml}$; nifedipine,

$3.54 \pm 0.46 \text{ pg/ml}$ —and were not affected by any of the interventions (Table 1). Baseline urine ET-1/creatinine levels were also similar in all three phases of the study: placebo, $761 \pm 95 \text{ ag/mmol}$; sitaxentan, $783 \pm 84 \text{ ag/mmol}$; nifedipine, $824 \pm 97 \text{ ag/mmol}$. Although placebo and nifedipine had no effect on urine ET-1/creatinine, sitaxentan reduced this variable by about 19% (Figure 1C). A similar effect was seen on urine ET-1 concentration (without correction to urine creatinine). Neither placebo nor nifedipine affected urine ET-1, whereas sitaxentan reduced this significantly (Table 1). An increase in urine ET-1/creatinine was associated with reductions in both proteinuria and BP (Figure 2, B and C; $r=0.41$; $P<0.05$ for both), and in multivariate analysis, the change in urine ET-1/creatinine independently predicted the changes in proteinuria and BP.

In addition to the important evidence of potentially renoprotective effects on proteinuria, BP, and arterial stiffness, the current data show that ET_A receptor antagonism selectively decreases serum uric acid, ADMA, and urinary ET-1 levels in patients with proteinuric CKD, independent of BP. These effects were seen in patients already receiving optimal treatment with angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers. These findings suggest a potential role for ET_A receptor antagonism in conferring additional longer-term cardiovascular and renal benefits in patients with CKD.

Table 1. Main study data at baseline and week 6 of each study period

Variable	Placebo		Sitaxentan		Nifedipine	
	Baseline	Week 6	Baseline	Week 6	Baseline	Week 6
24-hr proteinuria (g/d)	2.06 ± 0.38	2.00 ± 0.33	2.07 ± 0.34	1.46 ± 0.26^a	1.95 ± 0.30	1.99 ± 0.33
Protein-to-creatinine ratio (mg/mmol)	155 ± 31	153 ± 27	157 ± 28	114 ± 23^b	155 ± 27	152 ± 29
Mean arterial pressure (mmHg)	94.6 ± 2.2	94.3 ± 1.7	94.4 ± 1.8	90.7 ± 1.8^c	95.5 ± 2.0	91.7 ± 1.7^c
Systolic BP (mmHg)	125.4 ± 2.7	124.2 ± 1.9	124.3 ± 2.2	120.7 ± 1.9^c	125.7 ± 2.4	120.7 ± 1.6^c
Diastolic BP (mmHg)	77.9 ± 1.5	77.5 ± 1.2	77.9 ± 1.3	74.3 ± 1.3^a	78.9 ± 1.5	75.7 ± 1.2^a
PWV (m/s)	7.7 ± 0.3	8.0 ± 0.4	8.0 ± 0.3	7.6 ± 0.3^c	7.9 ± 0.3	7.6 ± 0.3^c
Central augmentation index (%)	20 ± 2	20 ± 2	20 ± 2	15 ± 2^a	19 ± 2	17 ± 2
Plasma ET-1 (pg/ml)	3.6 ± 0.5	3.7 ± 0.6	3.6 ± 0.5	3.7 ± 0.5	3.5 ± 0.5	3.5 ± 0.5
Urine ET-1 (pg/ml)	4.5 ± 0.4	4.7 ± 0.4	5.1 ± 0.4	4.2 ± 2.1^c	5.1 ± 0.4	4.7 ± 0.4

Values are given as predosing baseline \pm SEM.

^a $P<0.01$ for week 6 versus baseline.

^b $P=0.01$ for week 6 versus baseline.

^c $P<0.05$ for week 6 versus baseline.

Table 2. Renal substudy data from clearance studies performed at baseline and week 6 of each study period

Variable	Placebo		Sitaxentan		Nifedipine	
	Baseline	Week 6	Baseline	Week 6	Baseline	Week 6
GFR (ml/min)	56±7	54±8	57±8	48±8 ^a	59±8	58±9
Effective renal blood flow (ml/min)	533±66	552±65	511±63	543±73	562±82	530±72
Effective renal vascular resistance (mmHg/min per L)	230±52	206±39	236±44	232±48	248±58	254±56
Effective filtration fraction (%)	19.1±1.1	17.9±1.3	20.8±1.0	16.6±0.7 ^b	20.3±1.1	20.5±1.4

Values are given as predosing baseline ± SEM.

^a*P*<0.05 for sitaxentan at week 6 versus sitaxentan at baseline.

^b*P*<0.01 for sitaxentan at week 6 versus sitaxentan at baseline.

Decreasing serum uric acid may reduce cardiovascular risk and CKD progression.⁷ Treatment of asymptomatic hyperuricemia improves renal function²² and delays disease progression¹¹ in patients with early CKD (stage 3). In a different approach, withdrawal of the xanthine oxidase inhibitor allopurinol from a group of patients with stable CKD led to both worsening of hypertension and acceleration of renal dysfunction, although this occurred only in patients not taking an angiotensin-converting enzyme inhibitor.¹² However, these studies suggesting benefits of reducing serum uric acid used allopurinol as the therapeutic agent. More recently, the angiotensin-receptor blocker losartan has been shown to decrease serum uric acid in a group of patients with type 2 diabetes and nephropathy, and this reduction was associated with a reduction in CKD progression.²³ ET receptor antagonism offers a potentially novel approach to decreasing serum uric acid in patients with proteinuric CKD.

Only two studies have shown that ET receptor antagonism reduces serum uric acid, neither of which included patients with CKD. Six months of treatment with the selective ET_A receptor antagonist atrasentan reduced serum uric acid levels from 293 to 286 μmol/L in patients with early atherosclerosis.²⁴ Change in serum uric acid was not a primary endpoint in this study, and although this was a statistically significant reduction it is not clinically meaningful. In another small open-label study (*n*=15) in patients with pulmonary arterial hypertension and no control group, Ulrich and colleagues showed that 6 months' treatment with the mixed ET_{A/B} receptor

antagonist bosentan decreased serum uric acid from 353 to 305 μmol/L.²⁵ The current data build on these studies by showing that in a randomized controlled trial of patients with proteinuric CKD, in which baseline serum uric acid levels were much higher, selective ET_A receptor antagonism reduces serum uric acid by approximately 11% (more impressive reductions than seen in the previous two studies), independent of BP. Furthermore, as a mechanism for this we have shown an increase in the renal excretion of uric acid.

There is increasing interest in the NO system and, in particular, ADMA in relation to both the development and progression of CKD. Many studies in patients with varying degrees of CKD have confirmed that ADMA is elevated in CKD.^{14,26} Of note, data suggest that ADMA is elevated independently of renal function in CKD,²⁷ suggesting that mechanisms other than impaired clearance may contribute to the accumulation of ADMA in this setting. The ET system is upregulated in CKD.²⁰ There is often reciprocal upregulation of the ET system²⁰ in circumstances with downregulation of NO system activity.²⁰ In the current study, baseline plasma ADMA and ET-1 did indeed correlate highly with each other ($r^2=0.56$; *P*<0.01), confirming the reciprocal relationship between the NO and ET systems.

Few interventional studies have shown a reduction in ADMA. These have not included patients with CKD and have suffered from being small or lacking in rigorous methods.^{18,19} To our knowledge, the current study is the first to show that ET receptor antagonism may reduce circulating ADMA concentrations. We have previously shown in a

cross-sectional study that ADMA concentrations directly correlate with arterial stiffness—as measured by PVW—in a similar cohort of patients with CKD.²⁶ The current study takes this observation further by showing for the first time that a decrease in ADMA correlates with an improvement in arterial stiffness, although we recognize this correlation to be weak. Additionally, it is not possible to separate independent effects of the ET_A antagonist on arterial stiffness and ADMA in this limited number of patients. Because both increased ADMA and arterial stiffness independently contribute to CKD progression and its associated morbidity and mortality,^{14,20} ET receptor antagonism offers a potentially attractive novel therapy in CKD with benefits beyond those of lowering BP and proteinuria.

Urinary ET-1 is a recognized measure of renal ET-1 production.²⁸ Selective ET_A receptor antagonism reduced renal ET-1 production at 6 weeks. Of note, the decrease in BP seen with sitaxentan at 6 weeks correlated inversely (albeit weakly) with urinary ET-1; that is, a greater decrease in BP was seen in patients who had less of a reduction, or even an increase, in renal ET-1 production. Renal ET-1 is involved in salt and water excretion²⁹ and so part of the mechanism for the BP-lowering effect of ET receptor antagonism (in addition to their direct effects on the vasculature) may relate to an increase in renal ET-1 production to increase both natriuresis and diuresis. In this study there was no relationship between changes in salt excretion and urinary ET-1. A relationship similar to that seen with BP was also seen between the 6-week change in urinary ET-1 and

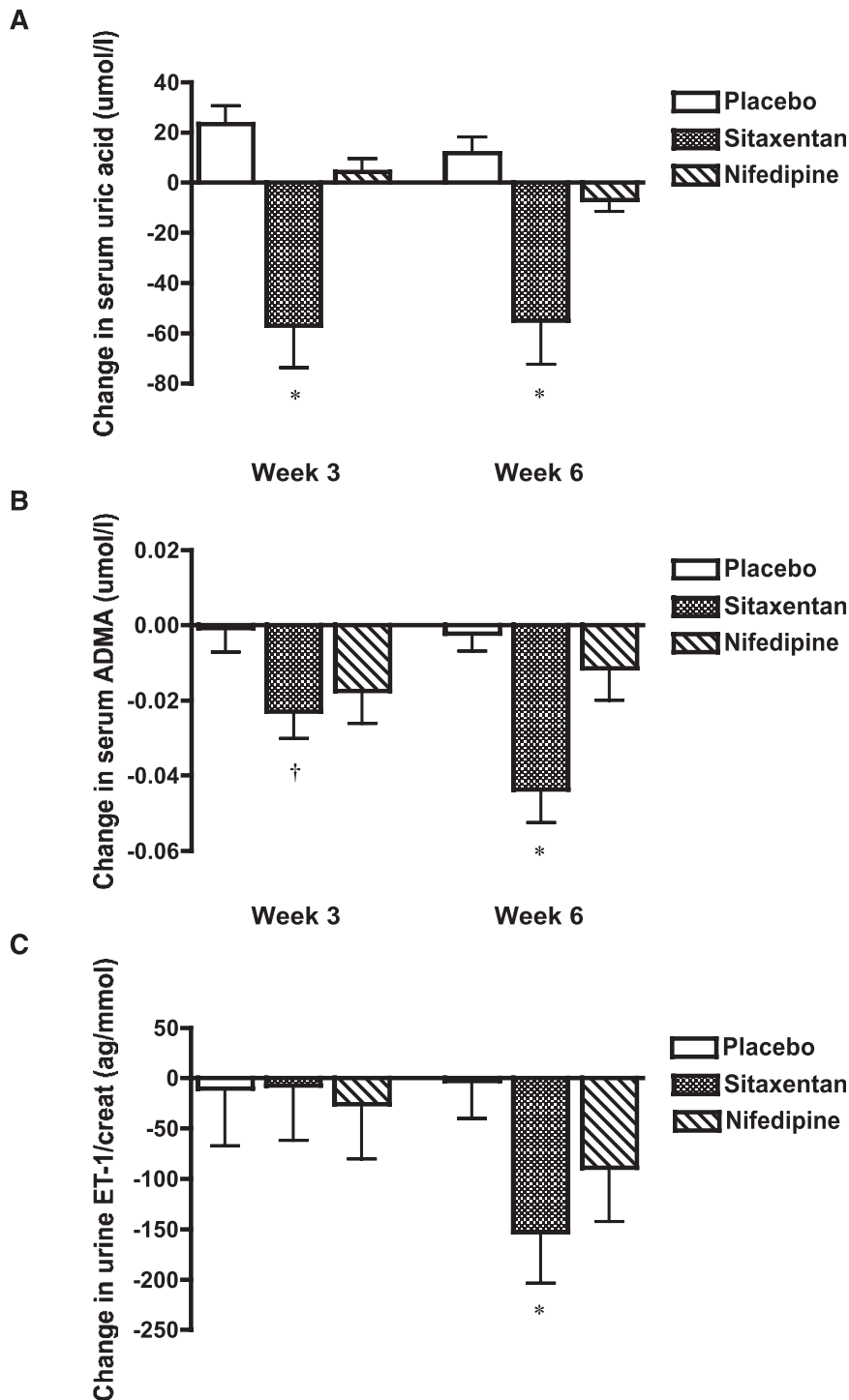


Figure 1. Selective endothelin-A receptor antagonism reduces serum urate, ADMA and urine ET-1/creatinine in CKD patients. Change from baseline in (A) serum uric acid, (B) ADMA, and (C) urine ET-1/creatinine after 3 and 6 weeks' treatment with placebo (open bar), sitaxentan (speckled bar), and nifedipine (hashed bar). Values are expressed as mean \pm SEM. * $P < 0.01$ for sitaxentan versus placebo at 3 or 6 weeks; † $P < 0.05$ for sitaxentan versus placebo at 3 weeks.

proteinuria. The reduction in proteinuria with sitaxentan related to the decrease in BP ($r^2=0.16$; $P=0.04$) and so this may explain part of this.

The current data show for the first time that selective ET_A receptor antagonism reduces novel cardiovascular risk factors in patients with proteinuric CKD established on optimal therapy. These data build on our earlier cross-sectional study, which showed that ADMA concentrations directly correlate with arterial stiffness, a powerful predictor of cardiovascular disease in patients with CKD. The mechanisms for these effects need to be further explored as a focus of future research. Certainly, reduction in BP is not sufficient because the active control agent nifedipine matched the decrease in BP seen with sitaxentan but did not reduce serum uric acid, ADMA, or urinary ET-1. Larger studies are needed to confirm these important findings in a group of patients at very high cardiovascular risk.

CONCISE METHODS

The rationale and design for this study have been reported in detail elsewhere.²¹ In brief, in a randomized, double-blind, three-way crossover study, 27 patients receiving recommended renoprotective treatment underwent 6 weeks of placebo; sitaxentan, 100 mg once daily; and nifedipine long-acting, 30 mg once daily. Twenty-four-hour proteinuria, urine protein-to-creatinine ratio, 24-hour ambulatory BP, and PWV (as an index of arterial stiffness) were measured at baseline, week 3, and week 6 of each treatment period. ADMA, serum urate, plasma ET-1, and urine ET-1/creatinine were also assessed at these same time points. See Supplemental Table for baseline patient characteristics.

Sample Collection and Analysis

ADMA and ET-1 venous blood samples were collected in EDTA tubes, and urate was collected in serum tubes. These were immediately centrifuged at 2500 g for 20 minutes at 4°C. For urine ET-1, a 20-ml aliquot of urine was collected into plain tubes with 2.5 ml of 50% acetic acid. Samples were stored at -80°C until analysis.

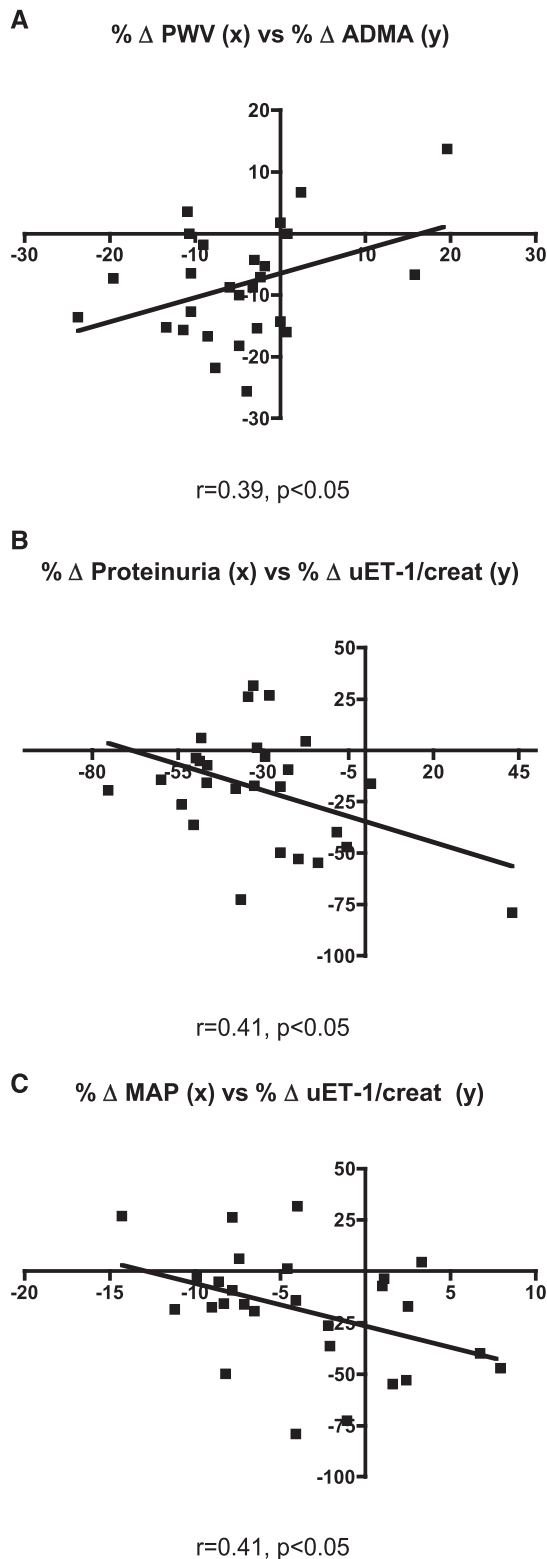


Figure 2. Changes in arterial stiffness, blood pressure and proteinuria following endothelin-A antagonism relate to changes in ADMA and urine ET-1/creat. Relationships between (A) percentage change in PWV against percentage change in ADMA and (B) percentage change in proteinuria and (C) percentage change in mean arterial pressure against percentage change in urine ET-1/creatinine. MAP, mean arterial pressure; uET-1/creat, urine ET-1/creatinine.

Plasma ADMA concentrations were measured using an optimized and fully validated HPLC method, as previously described³⁰ (assay variations, 1.9% and 2.3%).

After extraction,³¹ ET-1 was determined by radioimmunoassay.³² The mean recovery of ET-1, from extraction to assay, was >90% for both plasma and urine. The intra- and interassay variations were 6.3% and 7.2%, respectively. The cross-reactivity of the antibody was 100% with ET-1, 7% for both ET-2 and ET-3, and 10% with big ET-1.

The urate assay was based on the methods of Trivedi *et al.*³³ Uric acid is oxidized to allantoin by uricase with the production of hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and 2,4,6-tribromo-3-hydroxybenzoic acid in the presence of peroxidase to yield a quinoneimine dye. The resulting change in absorbance at 548 nm is proportional to the uric acid concentration in the sample. The limits of detection and quantification for the urate assay are 0.01 mmol/L and 0.015 mmol/L, respectively.

Statistical Analyses

Data were stored and analyzed in Microsoft Excel, version 11.3.7 (Microsoft Corp., Redmond, WA). Statistical analyses were performed on untransformed data. Responses were examined by repeated-measures ANOVA, and Bonferroni correction was used to assess significance at specific time points. Statistical significance was taken at the 5% level.

ADDENDUM

Sitaxentan has been voluntarily withdrawn by Pfizer Ltd. because of unacceptable adverse effects. However, the findings in this manuscript are likely to be representative of the effects of the class of selective ET_A receptor antagonists.

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