Endothelin antagonism for patients with chronic kidney disease: still a hope for the future

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ABSTRACT

Endothelin is tightly involved in the regulation of vascular and renal function in health and in disease. In a variety of animal models of kidney disease, endothelin promotes renal injury through effects on inflammation and fibrosis. Furthermore, experimental data strongly suggest that blocking the actions of endothelin should be beneficial in patients with chronic kidney disease. However, despite encouraging pre-clinical and clinical evidence, endothelin antagonists are not yet an established treatment option in patients with chronic kidney disease. This article reviews key physiological and pathophysiological aspects of the endothelin system in the vasculature and the kidney, as well as results of pre-clinical and clinical studies on the use of endothelin antagonists in chronic kidney disease. We will also provide an outlook on the future of endothelin antagonism in this area, and issues to be resolved before endothelin antagonists are to become a reality for patients with chronic kidney disease.

INTRODUCTION

More than 20 years ago, Yanagisawa *et al.* [1] discovered endothelin, the most powerful vasoconstrictor substance known in humans. In addition to its role in cardiovascular biology, the involvement of endothelin in many other physiological systems, which, to name a few, includes the nervous, the immune and the reproductive systems, has been firmly established. Endothelin is also involved in regulating the structure and function of the kidney, and experimental data strongly suggest that blocking the actions of endothelin might be beneficial in patients with chronic kidney disease (CKD). However, despite encouraging pre-clinical and clinical evidence, endothelin antagonists are not yet an established treatment option in patients with CKD. So far, approved clinical indications for endothelin antagonists are only pulmonary

arterial hypertension and scleroderma-related digital ulcers. This article reviews key physiological and pathophysiological aspects of the endothelin systems as relevant for CKD, as well as results of pre-clinical and clinical studies on the use of endothelin antagonists in CKD. We will also provide an outlook on the future of endothelin antagonism in CKD, and issues to be resolved before endothelin antagonists are to become a reality for patients with CKD.

PHYSIOLOGY OF THE VASCULAR ENDOTHELIN SYSTEM

Encoded by separate genes, the human endothelin family encompasses three 21-amino-acid long isopeptides, endothelin-1, endothelin-2 and endothelin-3 (ET-1, ET-2 and ET-3) [2]. In the cardiovascular and renal systems, the most extensively studied and most important isoform is ET-1. Endothelial cells are the major source of ET-1 in the vasculature (Figure 1). There are two types of receptors for endothelins: the endothelin type A (ET_A) and the endothelin type B (ET_B) receptor. Both ET_A and ET_B receptors, located on vascular smooth muscle cells, mediate strong and long-lasting vasoconstrictor responses to ET-1. The ET_B receptor, however, has two additional functions that tend to oppose the vasoconstrictor effects of ET-1. Activation of ET_B receptors on endothelial cells causes release of prostaglandins and nitric oxide, resulting in relaxation of the underlying smooth muscle cells. Further, endothelial ET_B receptors are important for clearing ET-1 from the circulation, thus limiting the vasoconstrictor activity of ET-1 (for a detailed discussion of the various roles of the ET_A and ET_B receptors, please see review [3]). In mice, endothelial-cell specific disruption of the ET-1 gene reduces blood pressure (BP), providing genetic evidence for an essential role of endothelium-derived ET-1 in the maintenance of basal vascular tone and BP, with an overall BP-increasing effect [4]. This is also the case in humans, as the overall effect of ET-1

FIGURE 1: The endothelin system in the vasculature.

FIGURE 2: The endothelin system in the kidney (inner renal medulla).

generated in the human vasculature is vasoconstriction [5]. In addition to the regulation of vascular tone, ET-1 also has growth-promoting effects on vascular smooth muscle cells.

PHYSIOLOGY OF THE RENAL ENDOTHELIN SYSTEM

In the kidney, the inner renal medulla, and in particular the principal cells of the inner medullary collecting duct (IMCD), produces the greatest amounts of ET-1 (Figure 2). Both ET_A and ET_B receptors are expressed in this region [6, 7]. Collecting duct cells express ET_A and ET_B receptors, pericytes of the vasa recta and smooth muscle cells of the more upstream afferent arterioles ET_A and ET_B receptors [8], and endothelial cells of the vasa recta and afferent arterioles ET_B receptors. Mice with collecting duct-specific deletion of ET-1 were found to have salt-sensitive hypertension, demonstrating the importance of ET-1 within this particular nephron segment for fluid balance and BP control [9, 10]. A further series of experiments with cell-specific deletions of the receptors showed that it is mainly the ET_B receptor on collecting duct cells that, in an autocrine fashion, mediates the natriuretic and antihypertensive effects of collecting duct-derived ET-1 [11–13]. Counteracting the vasoconstrictor actions of smooth muscle ET_A and ET_B receptors [8, 14], ET_B receptors on endothelial cells of afferent arterioles and the vasa recta contribute to the natriuretic and antihypertensive effects of ET-1 by triggering vasodilation and an increase in blood flow into the renal medulla. Increased medullary perfusion raises peritubular hydrostatic pressure and causes a reduction in the tonicity within the interstitium of the renal medulla. These, in turn, result in a decrease of sodium reabsorption along the

nephron. Interestingly, overall activation of ET_B receptors elicits afferent arteriolar constriction of juxtamedullary nephrons in rats during a normal salt intake, but dilation during a high salt intake [14, 15]. These findings suggest that endothelial ET_B receptors and preservation of medullary blood flow are antihypertensive mechanisms during high sodium diets. ET-1 has also been shown to inhibit sodium reabsorption in the thick ascending limb via actions through the ET_B receptor, which may contribute to the overall diuretic effects of ET-1 in the kidney [16, 17].

There is more recent evidence that under specific circumstances, ET_A receptors may also participate in the natriuretic and antihypertensive effects of renal medullary ET-1. Nephron-specific deletion of ET_A receptors in mice causes mild volume expansion under a high salt intake without a change of BP [18]. In female rats, ET_A receptors within the renal medulla contribute to ET-1-induced natriuresis by a nitric oxide synthase 1 (nNOS)-dependent mechanism, an effect that is abolished by ovariectomy [19]. Finally, there is recent evidence that ET-1 inhibits sodium reabsorption through both ET_A and ET_B receptors in the cortical collecting duct [20]. Therefore, summarizing the experimental data on the roles of the renal ET_A and ET_B receptors for renal sodium excretion, the activation of renal ET_B receptors clearly favours sodium excretion and thus contributes to a lower BP. The role of renal ET_A receptor is less clear, but recent experimental evidence suggests that the activation of those receptors may as well favour natriuresis, at least under specific circumstances.

PATHOPHYSIOLOGY OF THE ENDOTHELIN SYSTEM IN CKD

Alterations in the function and structure of mesangial cells and podocytes play an important pathogenetic role in the progression of renal diseases. Human mesangial cells and podocytes express functionally active endothelin receptors as they bind ET-1, which subsequently causes a rapid increase in intracellular calcium levels [21]. Thus, ET-1 significantly affects signal transduction and proliferation of mesangial cells and podocytes. There is also strong evidence that the activation of the endothelin system directly promotes renal fibrosis, as rats with transgenic overexpression of ET-1 develop severe glomerulosclerosis and interstitial fibrosis [22]. Cell culture studies have further shown that exposure to protein overload, as a general model of proteinuric nephropathies, and exposure to shigatoxin, as a model for the haemolytic uraemic syndrome, both upregulate ET-1 expression in cultured podocytes [23, 24]. The release of ET-1 then results in further deterioration of podocyte structure and function by an autocrine mechanism [23]. Experimental evidence for involvement of the endothelin system is particularly strong in diabetic kidney disease, as ET-1, the ET_A and ET_B receptors are strongly overexpressed in the renal cortex of rats with streptozotocin-induced diabetes [25].

In humans, there are only few data on the expression of the components of the endothelin system in patients with

kidney disease. For example, an increased expression of ET-1 has been found in kidneys of patients with IgA nephropathy [26, 27]. Some studies further suggest that urinary ET-1 levels may be a useful marker of renal injury [28, 29]. There is also some evidence from human studies that the ET_A receptor, and perhaps also the activation of the ET_B receptor, contribute to the progression of atherosclerosis, which is a significant clinical complication of CKD [30, 31].

EXPERIMENTAL STUDIES WITH ENDOTHELIN ANTAGONISTS

There is abundant evidence from experimental studies for the beneficial effects of endothelin antagonism in non-diabetic and diabetic models of renal disease. Ortmann *et al.* [32] have shown that 4-week oral treatment with the ET_A receptor selective antagonist darusentan partially reverses ageing-associated glomerulosclerosis in rats. Further experiments from that study demonstrated that the structural damage to podocytes induced by puromycin aminonucleoside, as a model of focal segmental glomerulosclerosis, is attenuated by ET_A receptor, but not ET_B receptor, blockade [32]. In the shigatoxin-induced model of the haemolytic uraemic syndrome, ET_A receptor blockade was able to prevent the pathological changes of the podocyte cytoskeleton [24]. Together, these studies support the concept that the activation of the endothelin system can contribute to renal disease progression, mediated partly via actions on podocytes.

Experimental data on whether selective ET_A receptor blockade should be preferred over non-selective ET_A/ET_B receptor blockade for preventing progressive renal disease are not entirely consistent. In models of non-diabetic renal disease, some studies, such as in the ageing-associated glomerulosclerosis model, suggest that ET_A selective blockade is more effective than non-selective ET_A/ET_B receptor blockade, whereas others, such as studies in the renal mass reduction model, suggest that treatment with both types of blockers are equally effective in protecting from disease progression [33, 34].

In the streptozotocin model of diabetes, selective ET_A receptor blockade has been shown to reduce albuminuria, extracellular matrix production and glomerular inflammation [35, 36]. Further studies have shown that selective ET_A receptor and non-selective ET_A/ET_B receptor blockade both reduce albuminuria and glomerular permeability, but only selective ET_A receptor blockade reduces glomerular inflammation [37]. Thus, selective ET_A receptor blockade may be preferable for treatment of diabetic kidney disease. Of note, in uninephrectomized rats with streptozotocin-induced diabetes, regression of glomerular and interstitial injury was achieved by ET_A receptor blockade with avosentan combined with renin-angiotensin system inhibition with lisinopril, whereas each drug alone was only able to attenuate glomerular and interstitial injury, suggesting synergistic effects of combining the two treatment principles [38]. Chronic ET_A receptor blockade has further been shown to normalize endothelial function and reduce atheroma formation in a mouse model of atherosclerosis, which, as alluded to earlier, presents a significant comorbidity in CKD [39].

CLINICAL STUDIES WITH ENDOTHELIN ANTAGONISTS

There was some evidence from early clinical experimental studies that the contribution of endothelins to vascular tone of the forearm vasculature is reduced (rather than increased) in patients with advanced CKD compared with healthy controls [40, 41]. Nonetheless, Goddard *et al.* [42] demonstrated that the ET_A selective antagonist BQ-123 significantly reduces BP and increases renal blood flow in hypertensive patients with non-diabetic CKD. In the same study, combined ET_A/ET_B receptor blockade also reduced BP, but was not able to increase renal blood flow. The renal haemodynamic effects of selective ET_A receptor blockade were similar to those commonly associated with renin-angiotensin system inhibition in that a reduction of filtration fraction was observed, perhaps suggesting similar renal protection in the long term [42]. Subsequent studies showed that ET_A receptor blockade with i.v. BQ-123 combined with renin-angiotensin system inhibition even had synergistic renal haemodynamic effects and together were able to increase sodium excretion significantly [43]. These synergistic effects were abolished with ET_B receptor blockade or with nNOS inhibition, suggesting that the beneficial effects of ET_A blockade were mediated via activation of the ET_B receptor. By way of mechanism, blockade of the ET_A receptor appears to leave more ET-1 available for activation of the ET_B receptor, i.e. ET-1 is shifted to activate more ET_B receptors [43]. Further, studies by Dhaun *et al.* [44] demonstrated that acute ET_A receptor blockade with i.v. BQ-123 reduces BP, arterial stiffness and proteinuria in patients with non-diabetic kidney disease. While the reduction in BP and the increase in renal blood flow were similar to the calcium channel blocker nifedipine—as a control—in comparison with BQ-123, there was a substantially greater reduction in arterial stiffness and proteinuria with BQ-123, suggesting BP-independent effects of ET_A receptor blockade.

In line with these acute effects ET_A receptor blockade, 6 weeks oral treatment with the sulphonamide-based, highly ET_A receptor selective antagonist sitaxentan reduced proteinuria more than oral treatment with nifedipine, despite similar reductions in BP [45]. Only sitaxentan reduced filtration fraction, suggesting that the disparate effects on proteinuria may at least in part be explained by differences in the renal haemodynamic effects of these two drugs. Interestingly, no cases of clinically significant oedema were reported with sitaxentan, which is highly selective for the ET_A receptor (>1000:1 ET_A:ET_B binding selectivity). Sitaxentan was approved for the treatment of pulmonary arterial hypertension in 2006. Increases in liver enzymes are relatively common with this and other endothelin antagonists (up to ≈10% of patients [46]). Unfortunately, several cases of fatal liver failure were reported with sitaxentan in the following years, and the drug was withdrawn from the market in 2010. It has been postulated that some endothelin antagonists, in particular bosentan and sitaxentan [47], cause hepatitis by inhibition of bile salt transporter pumps, but immune-mediated or idiosyncratic mechanisms are also possible.

Clinical trials in patients with type 2 diabetes and nephropathy have also been conducted with avosentan, a

sulphonamide-based, non-selective ET_A/ET_B receptor antagonist (50:1 ET_A:ET_B binding selectivity). An initial dose-finding study in 286 obese patients with diabetic nephropathy and relatively preserved kidney function (average estimated glomerular filtration rate of 80 mL/min) found substantial reductions in albuminuria by ~20–40%, again 'on top' of standard therapy with either an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin receptor blocker [48]. In the phase 3 trial that followed (ASCEND), patients with more advanced diabetic nephropathy were included (an average estimated glomerular filtration rate of 33 mL/min). Again, avosentan led to substantial reductions in albuminuria but the trial had to be terminated prematurely in 2007 as numerous patients suffered drug-related side effects including the development of severe heart failure, and there was a trend towards more deaths in the active treatment groups [49]. Most experts agree that heart failure is the consequence of fluid retention due to renal sodium retention, in particular with those endothelin antagonists that are less ET_A receptor selective.

Darusentan, a propanoic acid-based ET_A selective antagonist was studied in patients with resistant arterial hypertension, i.e. patients with arterial hypertension in which BP was not controlled by three antihypertensives including a diuretic. In a first dose-ranging study in 115 patients, adding darusentan lowered BP substantially [50]. In the second, larger study in 379 patients (DORADO), data on albuminuria were also presented. In addition to lowering BP, darusentan reduced urinary albumin excretion in those with proteinuric hypertensive nephropathy. Most frequent side effects were fluid retention and headaches. Unfortunately, despite these overall encouraging results and the potential of this drug in patients with proteinuric kidney disease, further development of darusentan was abandoned in December 2009.

Rabelink *et al.* were the first to report antiproteinuric effects with the non-sulphonamide, highly ET_A receptor selective antagonist atrasentan in a small exploratory study (1800:1 ET_A:ET_B binding selectivity). In 10 patients with type 1 diabetes treated for 12 weeks, potent antiproteinuric effects were seen on top of standard renin-angiotensin system blockade (thesis, University of Utrecht). Kohan *et al.* [51] have recently confirmed the potent antiproteinuric effects of atrasentan in a larger phase 2 trial in 89 obese patients with type 2 diabetes. A larger phase 3 trial to assess the efficacy of atrasentan in slowing the progression of renal function decline has just started.

Development of endothelin antagonists for patients with CKD has been slowed down by side effects of these drugs, which included oedema formation, heart failure and liver toxicity. Compared with the excellent safety profile of ACE inhibitors and angiotensin receptor blockers, these safety issues are considerable hurdles for pharmaceutical companies. Learning more about the biology of the endothelin system in health and in disease will perhaps make a safer use of these drugs possible in specific clinical conditions. As an example, oedema formation and subsequent heart failure appeared to be more frequent in those trials in which a less ET_A selective compound was used, which fits well with the experimental data that the renal ET_B receptor is mainly responsible for natriuresis. Since selectivity is also a matter of dose, using lower doses of ET_A

antagonists together with adequate use of diuretics may also help to overcome oedematous side effects in CKD patients. In terms of liver toxicity, inhibition of hepatobiliary transporters appears to differ significantly between specific endothelin antagonists, and we clearly need to learn more about the precise molecular mechanisms [47]. Of note, endothelin antagonists are teratogenic and will not be an option for women of childbearing potential [52].

In summary, endothelin antagonists have demonstrated impressive antiproteinuric effects in CKD patients, even in those with inhibition of the renin-angiotensin system. Since renin-angiotensin system inhibition can only provide partial protection from renal disease progression, further development of endothelin antagonists for patients with kidney disease still remains a hope for the future.

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CONFLICT OF INTEREST STATEMENT

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REFERENCES

1. Yanagisawa M, Kurihara H, Kimura S *et al.* A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; 332: 411–415
2. Inoue A, Yanagisawa M, Kimura S *et al.* The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci USA* 1989; 86: 2863–2867
3. Schneider MP, Boesen EI, Pollock DM. Contrasting actions of endothelin ET(A) and ET(B) receptors in cardiovascular disease. *Annu Rev Pharmacol Toxicol* 2007; 47: 731–759
4. Kisanuki YY, Emoto N, Ohuchi T *et al.* Low blood pressure in endothelial cell-specific endothelin 1 knockout mice. *Hypertension* 2010; 56: 121–128
5. Haynes WG, Ferro CJ, O'Kane KP *et al.* Systemic endothelin receptor blockade decreases peripheral vascular resistance and blood pressure in humans. *Circulation* 1996; 93: 1860–1870
6. Kohan DE. Endothelin synthesis by rabbit renal tubule cells. *Am J Physiol* 1991; 261: F221–F226
7. Kohan DE, Hughes AK, Perkins SL. Characterization of endothelin receptors in the inner medullary collecting duct of the rat. *J Biol Chem* 1992; 267: 12336–12340
8. Silldorff EP, Yang S, Pallone TL. Prostaglandin E2 abrogates endothelin-induced vasoconstriction in renal outer medullary descending vasa recta of the rat. *J Clin Invest* 1995; 95: 2734–2740
9. Ahn D, Ge Y, Stricklett PK *et al.* Collecting duct-specific knockout of endothelin-1 causes hypertension and sodium retention. *J Clin Invest* 2004; 114: 504–511
10. Schneider MP, Ge Y, Pollock DM *et al.* Collecting duct-derived endothelin regulates arterial pressure and Na excretion via nitric oxide. *Hypertension* 2008; 51: 1605–1610
11. Ge Y, Bagnall A, Stricklett PK *et al.* Collecting duct-specific knockout of the endothelin B receptor causes hypertension and sodium retention. *Am J Physiol Renal Physiol* 2006; 291: F1274–F1280

12. Ge Y, Bagnall A, Stricklett PK *et al.* Combined knockout of collecting duct endothelin A and B receptors causes hypertension and sodium retention. *Am J Physiol Renal Physiol* 2008; 295: F1635–F1640
13. Ge Y, Stricklett PK, Hughes AK *et al.* Collecting duct-specific knockout of the endothelin A receptor alters renal vasopressin responsiveness, but not sodium excretion or blood pressure. *Am J Physiol Renal Physiol* 2005; 289: F692–F698
14. Inscho EW, Imig JD, Cook AK *et al.* ETA and ETB receptors differentially modulate afferent and efferent arteriolar responses to endothelin. *Br J Pharmacol* 2005; 146: 1019–1026
15. Schneider MP, Inscho EW, Pollock DM. Attenuated vasoconstrictor responses to endothelin in afferent arterioles during a high-salt diet. *Am J Physiol Renal Physiol* 2007; 292: F1208–F1214
16. Plato CF, Pollock DM, Garvin JL. Endothelin inhibits thick ascending limb chloride flux via ET(B) receptor-mediated NO release. *Am J Physiol Renal Physiol* 2000; 279: F326–F333
17. Herrera M, Hong NJ, Ortiz PA *et al.* Endothelin-1 inhibits thick ascending limb transport via Akt-stimulated nitric oxide production. *J Biol Chem* 2009; 284: 1454–1460
18. Stuart D, Rees S, Woodward SK *et al.* Disruption of the endothelin A receptor in the nephron causes mild fluid volume expansion. *BMC Nephrol* 2012; 13: 166
19. Nakano D, Pollock DM. Contribution of endothelin A receptors in endothelin 1-dependent natriuresis in female rats. *Hypertension* 2009; 53: 324–330
20. Lynch IJ, Welch AK, Kohan DE *et al.* Endothelin-1 inhibits sodium reabsorption by ETA and ETB receptors in the mouse cortical collecting duct. *Am J Physiol Renal Physiol* 2013 May 22. [Epub ahead of print]
21. Rebibou JM, He CJ, Delarue F *et al.* Functional endothelin 1 receptors on human glomerular podocytes and mesangial cells. *Nephrol Dial Transplant* 1992; 7: 288–292
22. Hoher B, Thone-Reineke C, Rohmeiss P *et al.* Endothelin-1 transgenic mice develop glomerulosclerosis, interstitial fibrosis, and renal cysts but not hypertension. *J Clin Invest* 1997; 99: 1380–1389
23. Morigi M, Buelli S, Angioletti S *et al.* In response to protein load podocytes reorganize cytoskeleton and modulate endothelin-1 gene: implication for permesective dysfunction of chronic nephropathies. *Am J Pathol* 2005; 166: 1309–1320
24. Morigi M, Buelli S, Zanchi C *et al.* Shigatoxin-induced endothelin-1 expression in cultured podocytes autocrinally mediates actin remodeling. *Am J Pathol* 2006; 169: 1965–1975
25. Minchenko AG, Stevens MJ, White L *et al.* Diabetes-induced overexpression of endothelin-1 and endothelin receptors in the rat renal cortex is mediated via poly(ADP-ribose) polymerase activation. *FASEB J* 2003; 17: 1514–1516
26. Rastaldi MP, Tunesi S, Ferrario F *et al.* Transforming growth factor- β , endothelin-1, and c-fos expression in necrotizing/crescentic IgA glomerulonephritis. *Nephrol Dial Transplant* 1998; 13: 1668–1674
27. Lehrke I, Waldherr R, Ritz E *et al.* Renal endothelin-1 and endothelin receptor type B expression in glomerular diseases with proteinuria. *J Am Soc Nephrol* 2001; 12: 2321–2329
28. Ohta K, Hirata Y, Shichiri M *et al.* Urinary excretion of endothelin-1 in normal subjects and patients with renal disease. *Kidney Int* 1991; 39: 307–311
29. Dhaun N, Lilithkarntakul P, Macintyre IM *et al.* Urinary endothelin-1 in chronic kidney disease and as a marker of disease activity in lupus nephritis. *Am J Physiol Renal Physiol* 2009; 296: F1477–F1483
30. Iwasa S, Fan J, Shimokama T *et al.* Increased immunoreactivity of endothelin-1 and endothelin B receptor in human atherosclerotic lesions. A possible role in atherogenesis. *Atherosclerosis* 1999; 146: 93–100
31. Bacon CR, Cary NR, Davenport AP. Endothelin peptide and receptors in human atherosclerotic coronary artery and aorta. *Circ Res* 1996; 79: 794–801
32. Ortmann J, Amann K, Brandes RP *et al.* Role of podocytes for reversal of glomerulosclerosis and proteinuria in the aging kidney after endothelin inhibition. *Hypertension* 2004; 44: 974–981
33. Benigni A, Zoja C, Corna D *et al.* A specific endothelin subtype A receptor antagonist protects against injury in renal disease progression. *Kidney Int* 1993; 44: 440–444
34. Benigni A, Zola C, Corna D *et al.* Blocking both type A and B endothelin receptors in the kidney attenuates renal injury and prolongs survival in rats with remnant kidney. *Am J Kidney Dis* 1996; 27: 416–423
35. Nakamura T, Ebihara I, Fukui M *et al.* Effect of a specific endothelin receptor A antagonist on mRNA levels for extracellular matrix components and growth factors in diabetic glomeruli. *Diabetes* 1995; 44: 895–899
36. Sasser JM, Sullivan JC, Hobbs JL *et al.* Endothelin A receptor blockade reduces diabetic renal injury via an anti-inflammatory mechanism. *J Am Soc Nephrol* 2007; 18: 143–154
37. Saleh MA, Pollock JS, Pollock DM. Distinct actions of endothelin A-selective versus combined endothelin A/B receptor antagonists in early diabetic kidney disease. *J Pharmacol Exp Ther* 2011; 338: 263–270
38. Gagliardini E, Corna D, Zoja C *et al.* Unlike each drug alone, lisinopril if combined with avosentan promotes regression of renal lesions in experimental diabetes. *Am J Physiol Renal Physiol* 2009; 297: F1448–F1456
39. Barton M, Haudenschild CC, d'Uscio LV *et al.* Endothelin ETA receptor blockade restores NO-mediated endothelial function and inhibits atherosclerosis in apolipoprotein E-deficient mice. *Proc Natl Acad Sci USA* 1998; 95: 14367–14372
40. Bussemaker E, Passauer J, Reimann D *et al.* The vascular endothelin system is not overactive in normotensive hemodialysis patients. *Kidney Int* 2002; 62: 940–948
41. Hand MF, Haynes WG, Webb DJ. Reduced endogenous endothelin-1-mediated vascular tone in chronic renal failure. *Kidney Int* 1999; 55: 613–620
42. Goddard J, Johnston NR, Hand MF *et al.* Endothelin-A receptor antagonism reduces blood pressure and increases renal blood flow in hypertensive patients with chronic renal failure: a comparison of selective and combined endothelin receptor blockade. *Circulation* 2004; 109: 1186–1193
43. Goddard J, Eckhart C, Johnston NR *et al.* Endothelin A receptor antagonism and angiotensin-converting enzyme inhibition are synergistic via an endothelin B receptor-mediated and nitric oxide-dependent mechanism. *J Am Soc Nephrol* 2004; 15: 2601–2610
44. Dhaun N, Macintyre IM, Melville V *et al.* Blood pressure-independent reduction in proteinuria and arterial stiffness after acute endothelin-a receptor antagonism in chronic kidney disease. *Hypertension* 2009; 54: 113–119
45. Dhaun N, MacIntyre IM, Kerr D *et al.* Selective endothelin-A receptor antagonism reduces proteinuria, blood pressure, and arterial stiffness in chronic proteinuric kidney disease. *Hypertension* 2011; 57: 772–779
46. Humbert M, Segal ES, Kiely DG *et al.* Results of European post-marketing surveillance of bosentan in pulmonary hypertension. *Eur Respir J* 2007; 30: 338–344
47. Hartman JC, Brouwer K, Mandagere A *et al.* Evaluation of the endothelin receptor antagonists ambrisentan, darusentan, bosentan, and sitaxsentan as substrates and inhibitors of hepatobiliary transporters in sandwich-cultured human hepatocytes. *Can J Physiol Pharmacol* 2010; 88: 682–691
48. Wenzel RR, Littke T, Kuranoff S *et al.* Avosentan reduces albumin excretion in diabetics with macroalbuminuria. *J Am Soc Nephrol* 2009; 20: 655–664
49. Mann JF, Green D, Jamerson K *et al.* Avosentan for overt diabetic nephropathy. *J Am Soc Nephrol* 2010; 21: 527–535
50. Black HR, Bakris GL, Weber MA *et al.* Efficacy and safety of darusentan in patients with resistant hypertension: results from a randomized, double-blind, placebo-controlled dose-ranging study. *J Clin Hypertens (Greenwich)* 2007; 9: 760–769
51. Kohan DE, Pritchett Y, Molitch M *et al.* Addition of atrasentan to renin-angiotensin system blockade reduces albuminuria in diabetic nephropathy. *J Am Soc Nephrol* 2011; 22: 763–772
52. Cross DM, Horsley E, Derzi M *et al.* An evaluation of reproductive and developmental toxicity of sitaxentan (thelin) in rats. *Birth Defects Res B Dev Reprod Toxicol* 2012; 95: 327–336

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