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

Markus P. Schneider¹ and Johannes F. Mann¹,²

¹Department of Nephrology and Hypertension, University of Erlangen-Nuremberg and Nuremberg General Hospital, Erlangen, Germany and ²Department of Nephrology, Hypertension & Rheumatology, Munich General Hospitals, Munich, Germany

Correspondence and offprint requests to: Markus P. Schneider; E-mail: markus.schneider@uk-erlangen.de

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 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.

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 (ETₐ) and the endothelin type B (ET₇) receptor. Both ETₐ and ET₇ receptors, located on vascular smooth muscle cells, mediate strong and long-lasting vasoconstrictor responses to ET-1. The ET₇ receptor, however, has two additional functions that tend to oppose the vasoconstrictor effects of ET-1. Activation of ET₇ receptors on endothelial cells causes release of prostaglandins and nitric oxide, resulting in relaxation of the underlying smooth muscle cells. Further, endothelial ET₇ 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 ETₐ and ET₇ 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

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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 ETA and ETB receptors are expressed in this region [6, 7]. Collecting duct cells express ETA and ETB receptors, pericytes of the vasa recta and smooth muscle cells of the more upstream afferent arterioles ETA and ETB receptors [8], and endothelial cells of the vasa recta and afferent arterioles ETB 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 ETB receptor on collecting duct cells that, in an autocrine fashion, mediates the natriuretic and antihypertensive effects of renal medullary ET-1. Nephron-specific deletion of ETA receptors in mice causes mild volume expansion under a high salt intake without a change of BP [18]. In female rats, ETB 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 ETA and ETB receptors in the cortical collecting duct [20]. Therefore, summarizing the experimental data on the roles of the renal ETA and ETB receptors for renal sodium excretion, the activation of renal ETB receptors clearly favours sodium excretion and thus contributes to a lower BP. The role of renal ETA 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 ETs receptors on endothelial cells of the vasa recta and smooth muscle cells of the more upstream afferent arterioles ETA and ETB receptors [8], and ETB receptors on endothelial cells of the vasa recta and afferent arterioles ETB 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 ETB receptor on collecting duct cells that, in an autocrine fashion, mediates the natriuretic and antihypertensive effects of renal medullary ET-1. Nephron-specific deletion of ETA receptors in mice causes mild volume expansion under a high salt intake without a change of BP [18]. In female rats, ETB 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 ETA and ETB receptors in the cortical collecting duct [20]. Therefore, summarizing the experimental data on the roles of the renal ETA and ETB receptors for renal sodium excretion, the activation of renal ETB receptors clearly favours sodium excretion and thus contributes to a lower BP. The role of renal ETA 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.

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 ET₄ receptor, and perhaps also the activation of the ET₃ 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 ET₄ 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 ET₄ receptor, but not ET₅ receptor, blockade [32]. In the shigatoxin-induced model of the haemolytic uraemic syndrome, ET₄ 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 ET₄ receptor blockade should be preferred over non-selective ET₄/ET₅ 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 ET₄ selective blockade is more effective than non-selective ET₄/ET₅ 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 ET₄ receptor blockade has been shown to reduce albuminuria, extracellular matrix production and glomerular inflammation [35, 36]. Further studies have shown that selective ET₄ receptor and non-selective ET₄/ET₅ receptor blockade both reduce albuminuria and glomerular permeability, but only selective ET₄ receptor blockade reduces glomerular inflammation [37]. Thus, selective ET₄ 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 ET₄ 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 ET₄ 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 ET₄ selective antagonist BQ-123 significantly reduces BP and increases renal blood flow in hypertensive patients with non-diabetic CKD. In the same study, combined ET₄/ET₅ receptor blockade also reduced BP, but was not able to increase renal blood flow. The renal haemodynamic effects of selective ET₄ 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 ET₄ 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 ET₅ receptor blockade or with nNOS inhibition, suggesting that the beneficial effects of ET₄ blockade were mediated via activation of the ET₅ receptor. By way of mechanism, blockade of the ET₄ receptor appears to leave more ET-1 available for activation of the ET₅ receptor, i.e. ET-1 is shifted to activate more ET₅ receptors [43]. Further, studies by Dhaun et al. [44] demonstrated that acute ET₄ 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 ET₄ receptor blockade.

In line with these acute effects ET₄ receptor blockade, 6 weeks oral treatment with the sulphonamide-based, highly ET₄ 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 ET₄ receptor (>1000:1 ET₄/ET₅ 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 \( \text{ET}_A/\text{ET}_B \) receptor antagonist \((50:1 \ \text{ET}_A:\text{ET}_B \) 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 \( \sim 20–40\% \), 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 \( \text{ET}_A \) receptor selective.

Darusentan, a propanoic acid-based \( \text{ET}_A \) 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 \( \text{ET}_A \) receptor selective antagonist atrasentan in a small exploratory study \((1800:1 \ \text{ET}_A: \text{ET}_B \) 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 \( \text{ET}_A \) selective compound was used, which fits well with the experimental data that the renal \( \text{ET}_B \) receptor is mainly responsible for natriuresis. Since selectivity is also a matter of dose, using lower doses of \( \text{ET}_A \) 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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