Endothelin antagonists for diabetic and non-diabetic chronic kidney disease

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Numerous pre-clinical studies have implicated endothelin-1 in the pathogenesis of diabetic and non-diabetic chronic kidney disease (CKD). Renal endothelin-1 production is almost universally increased in kidney disease. The pathologic effects of endothelin-1, including vasoconstriction, proteinuria, inflammation, cellular injury and fibrosis, are likely mediated by the endothelin A (ETA) receptor. ETA antagonism alone, and/or combined ETA/B blockade, reduces CKD progression. Based on the strong pre-clinical data, several clinical trials using ETA antagonists were conducted. Small trials involving acute intravenous endothelin receptor blockade suggest that ETA, but not ETB, blockade exerts protective renal and vascular effects in CKD patients. A large phase 3 trial (ASCEND) examined the effects of avosentan, an endothelin receptor antagonist, on renal disease progression in diabetic nephropathy. Proteinuria was reduced after 3–6 months of treatment. However the study was terminated due to increased morbidity and mortality associated with avosentan-induced fluid retention. Several phase 2 trials using avosentan at lower doses than in ASCEND, atrasentan or sitaxsentan (the latter two being highly ETA-selective) showed reductions in proteinuria on top of renin-angiotensin system blockade. Infrequent and clinically insignificant fluid retention was observed at the most effective doses. Additional trials using ETA blockers are ongoing or being planned in patients with diabetic nephropathy or focal segmental glomerulosclerosis. Moving forward, such studies must be conducted with careful patient selection and attention to dosing in order to minimize adverse side effects. Nonetheless, there is cause for optimism that this class of agents will ultimately prove to be effective for the treatment of CKD.

Introduction

Endothelin-1 (ET-1) is a 21 amino acid peptide that has a wide range of autocrine and paracrine actions within the kidney through activation of two receptor subtypes, ETA and ETB [1]. Activation of ETA receptors in vascular smooth muscle causes extremely potent vasoconstriction, while activation of ETB receptors in vascular endothelium induces vasorelaxation via nitric oxide and prostaglandin release. ETB receptors also reduce arterial pressure by promoting a natriuresis and diuresis through direct inhibition of nephron sodium and water reabsorption [1]. Under normal physiologic conditions, ETB receptor activation may be of primary importance in regulation of arterial pressure. When an ETA selective antagonist is given to animals or humans, no or only a modest decrease in arterial pressure occurs [1, 2]. However, an ETB receptor selective antagonist elicits a profound increase in arterial pressure (e.g. up to 40–50 mmHg in Sprague-Dawley rats depending on the salt intake) [3].

The ET system is located throughout the nephron and renal vasculature. Details of the renal ET system can be found in a recent review [1]. In general, ET-1 is synthesized by the vascular endothelium, mesangial cells and tubular epithelium, with the largest amount being produced by the collecting duct. Similarly, ETA and ETB receptors are expressed in renal tubular and vascular elements. ETB receptors are found in the greatest abundance within the inner medullary collecting duct. There is some variability amongst species, but approximately 30% of ET receptors expressed in the human renal cortex and medulla are ETA [4].

Role of endothelin in chronic kidney disease: experimental models

Similar to the vasculature, the balance between ETA and ETB receptor activation determines the effects of ET-1

within the kidney, particularly in disease states. In hypertensive models such as the Dahl salt-sensitive rat or the chronic angiotensin II-infused rat, ETB receptor function is reduced, increasing ETA receptor activity, and thus a clear antihypertensive effect of ETA receptor blockers is observed [5, 6]. In addition to increasing arterial pressure, ET-1 promotes renal inflammation, oxidative stress, vascular shear stress and hypoxia [1]. Each of these factors also increases renal ET-1 production, thereby forming a vicious cycle. The pathologic effects of ET-1 in the kidney are largely mediated by activation of the ETA receptor. ETA receptor blockade can reduce renal injury and inflammation through lowering of arterial pressure as well as directly reducing renal injury [7, 8]. Little is known about changes in ET receptor function associated with renal disease although recent evidence suggests a loss of ETB-dependent natriuresis when the renin-angiotensin system is activated [5, 9].

There have been many studies in animal models attempting to discern the role of ET-1 in acute and chronic kidney disease. Most studies examining the effects of selective ETA or combined ETA/B receptor antagonists have observed some degree of renal protection [10, 11]. As stated above, in addition to the potent renal vasoconstriction that could contribute to a decline in blood flow and glomerular filtration rate (GFR), ET-1 also increases oxidative stress and inflammation within the kidney. Members of our investigative team have provided pre-clinical evidence for both of these mechanisms contributing to long term decline in renal function [8, 12, 13].

Proteinuria represents an early sign of glomerular injury and its presence predicts not only an elevated risk for nephropathy, but also generalized cardiovascular disease. ETA receptors likely play a role in development of proteinuria, being located on mesangial and endothelial cells as well as podocytes [14]. Studies from several groups have shown that chronic blockade of ETA receptors reduces albuminuria in the diabetic rat independent of lowering of arterial pressure [13, 15]. The mechanism of ET-1-induced albuminuria appears to be due, at least in part, to a direct effect on the ETA receptor to increase glomerular permeability to albumin [8, 16].

ETA receptor blockade has also been shown to reduce renal inflammation in both the diabetic and hypertensive kidney. Inflammation is considered to be a contributing factor in the development of CKD and may contribute to proteinuria as well as interstitial fibrosis and cellular damage. Pro-inflammatory chemokines such as monocyte chemoattractant protein-1, vascular cell adhesion molecule-1 (VCAM-1) and soluble inter-cellular adhesion molecule-1 (sICAM-1) promote macrophage infiltration into the kidney. Increased ICAM-1 and/or VCAM-1 expression leading to increased leukocyte infiltration has been demonstrated in experimental models of nephropathy [17]. Our group has recently published that ET-1, via the ETA receptor, directly increases expression of these

early inflammatory markers in the kidney independent of hypertension [8].

Preclinical studies have provided considerable proof of concept data regarding the use of ET antagonists for the treatment of kidney disease in general, and diabetic nephropathy in particular [13, 18–20]. These studies used a range of different antagonists with varying selectivity for ETA and/or ETB receptors. Hocher and colleagues [21] were among the first to demonstrate that ETA selective blockade reduced renal injury and improved function beyond what could be seen with ACE inhibition. However, this benefit beyond ACE inhibition on proteinuria has not consistently been observed [22]. Watson *et al.* reported that avosentan, an antagonist with very modest ETA selectivity, reduced proteinuria and glomerular injury in apo-E knockout mice with type 1 diabetes [19]. As in the Hocher *et al.* study, they also reported improvements in glomerular injury beyond that observed with an ACE inhibitor. Using avosentan in a streptozotocin model, Gagliardini and colleagues [18] demonstrated that the combination of ET receptor blockade and ACE inhibition produced additional benefit beyond single agent therapy, and in fact, suggested that this treatment regimen may cause regression of established injury. These results complement studies by Saleh *et al.* in the same model where the more selective ETA antagonist, atrasentan, improved glomerular permeability [16, 23]. Zoja and colleagues have also observed reduced proteinuria and renal injury in the Zucker fatty diabetic rat using the selective antagonist, sitaxsentan, and again demonstrated beneficial effects beyond that of ACE inhibition alone [20].

The question of whether combined ETA and ETB antagonism as compared with a selective ETA antagonist would be preferable in treating CKD has been a matter of debate. In general, ETA-selective antagonists have consistently proven to improve renal function in various animal models of CKD. Some animal data have also shown that a non-selective antagonist, such as bosentan, can produce beneficial effects in a diabetic kidney disease model [24]. Saleh and colleagues directly compared a combined vs. ETA selective antagonist in the type 1 diabetic rat model and observed that while the ETA selective antagonist produced a more rapid decline in proteinuria, after 1 week treatment the decline in proteinuria was similar between the two types of antagonists [16]. However, only the ETA antagonist had anti-inflammatory and anti-fibrotic effects. In addition, ETB blockade increased disease progression in a model of polycystic kidney disease and this was prevented by ETA blockade [25]. Furthermore, combined ETA/B blockade in ischaemic acute renal failure increased long term renal injury, while selective ETA blockade was beneficial [26]. Thus, while it remains to be determined whether there is any real benefit to targeting ETA vs. both ETA and ETB, the available evidence suggests that ETA-selective blockade might be advantageous.

Endothelin antagonists in CKD: clinical studies

Increased urinary ET-1 excretion is almost universally seen in CKD and reflects elevated renal ET-1 production associated with renal injury [27, 28]. Plasma ET-1 is also increased in patients with CKD and correlates with urinary albumin excretion and severity of renal functional impairment [29, 30]. The elevated plasma ET-1 is likely due to reduced renal ET-1 clearance, a pro-inflammatory state, acidosis and vascular injury. With regard to acidosis, bicarbonate treatment decreased renal ET-1 production and the rate of GFR decline in CKD patients with low or moderately reduced GFR [31, 32]. With regard to vascular injury, plasma ET-1 concentrations in CKD patients are independent predictors of reduced flow-mediated brachial artery dilatation and increased carotid-femoral pulse wave velocity (PWV) [33]. These increases in ET-1, combined with the experimental model data, have provided a strong impetus for the use of ET receptor antagonists (ERAs) in CKD.

Initial clinical studies on the role of ERAs in treating CKD involved acute drug administration. Intravenous infusion of BQ123, an ETA receptor-selective ERA, reduced BP and increased renal blood flow in eight hypertensive CKD patients, yet did not affect the renal circulation in healthy controls [34]. This suggested that renal vascular ETA receptors are functionally overactive in CKD patients. In contrast, BQ788, an ETB-selective ERA, caused systemic and renal vasoconstriction when given alone, and prevented BQ123-induced renal vasodilation. This suggested that ETB blockade, insofar as renal vascular function was concerned, may be disadvantageous in CKD patients. In a follow-up study, BQ123 acutely reduced proteinuria and PWV in 22 non-diabetic CKD patients, and this effect was greater than that of the active comparator, nifedipine [35]. A later analysis of this data revealed that BQ123-induced reductions in proteinuria and PWV were greater in individuals taking combined angiotensin converting inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), as compared with ACEIs alone, supporting the notion that ERAs have a potential protective renal role in the setting of maximal renin-angiotensin system (RAS) blockade [36]. Finally, this same group reported that intravenous infusion of TAK-044, a relatively non-selective ERA (~17-fold greater affinity for ETA as compared with ETB receptors), to seven non-diabetic CKD patients reduced BP and tended to increase renal plasma flow [37]. Taken together, these findings using acute ERA administration suggest that ETA blockade exerts protective renal and vascular effects in CKD patients (the choice of which ET receptor to target in CKD is reviewed in detail in [38]).

The first phase 2 trial involving ERAs in CKD, conducted by Speedel, studied the effect of 12 weeks of avosentan, a relatively ETA-selective antagonist (ETA:B

blockade ~300:1), on urinary mean albumin excretion rate (UAER) [39]. This study involved 286 patients with diabetic nephropathy on RAS blockade with a creatinine clearance of ~80 ml min⁻¹ and UAER of ~1500 mg day⁻¹. Avosentan at doses of 5, 10, 25 and 50 mg day⁻¹ decreased UACR by 20.9, 16.3, 25.0 and 29.9%, respectively; the placebo group had an unexpected increase in UACR (35.5%). The authors claimed this was a dose-dependent response. However it is questionable whether such dose-dependency really existed given the small differences between groups. The only significant side effect observed was fluid retention. There were more episodes of fluid retention at the highest dose (11.9, 21.1, 15.0 and 32.1% in the 5, 10, 25 and 50 mg day⁻¹ groups, respectively). Based on their interpretation of these data, Speedel launched a phase 3 trial (ASCEND) examining the effect of avosentan on renal disease progression or death in type II diabetic nephropathy patients on RAS blockade [40]. A total of 1392 individuals from 36 countries were given 25 or 50 mg day⁻¹ of avosentan or placebo. Enrollees had a median albumin to creatinine ratio (ACR) at baseline of ~1500 mg g⁻¹ and an eGFR of ~33 ml min⁻¹ 1.73 m⁻². Avosentan reduced the ACR (44.3, 49.3 and 9.7% for the 25 mg, 50 mg and placebo groups, respectively). However, the trial was prematurely terminated due to greater serious adverse cardiovascular events in the avosentan groups, including a three-fold increase in the incidence of congestive heart failure. It is unclear why the 25 and 50 mg avosentan doses were used, given that lower doses may have comparable efficacy in reducing albuminuria, while causing less fluid retention. Such high doses of avosentan may have affected the ETB receptor, an effect that would be predicted to promote additional fluid retention [1]. Furthermore, the ASCEND trial involved patients with advanced kidney disease who may have been more likely to retain fluid.

The failure of the ASCEND trial underscored the importance of careful patient selection and ERA dosing in CKD. A subsequent study evaluated the effect of 0.25, 0.75 or 1.75 mg day⁻¹ atrasentan (ETA:B receptor selectivity of ~1200:1) or placebo for 8 weeks on the ACR in 89 subjects with diabetic nephropathy (baseline ACR 350–515 mg g⁻¹ and eGFR 48–61 ml min⁻¹ 1.73 m⁻²) receiving stable doses of RAS inhibitors [2]. Atrasentan reduced the ACR in the 0.75 mg and 1.75 mg groups as compared with placebo (42, 35% vs. 11% decreases, respectively). The reduction in ACR was evident after 1 week of treatment and was associated with a fall in BP, suggesting that the initial anti-proteinuric effect of atrasentan may be haemodynamic-related. The only adverse event was mild to moderate peripheral edema that was dose-related: 9, 14, 18 and 46% in placebo and the 0.25, 0.5 and 1.75 mg atrasentan groups, respectively. Thus, an antiproteinuric dose of an ERA was identified at which minimal fluid retention occurred, once again underscoring the importance of

careful study design. In a second study, sitaxsentan, a highly selective ETA blocker (ETA : B selectivity of ~6000 : 1) was given to 27 non-diabetic CKD (stages 1–4) subjects for 6 weeks [41]. Sitaxsentan, but not the active comparator nifedipine, reduced proteinuria, while no clinically significant adverse events occurred. Together, these studies rekindled hope for using ERAs in treating patients with CKD.

Currently, several trials involving ETA-selective antagonists in CKD are active or in the development stages. Three trials involving atrasentan administration to patients with diabetic nephropathy are ongoing: (i) a phase 2b 12 week trial evaluating the effect of low or high dose atrasentan on residual albuminuria in patients ($n=150$) on maximal tolerated RAS blockade (eGFR 30–75 ml min⁻¹ 1.73 m⁻², ACR >300–3500 mg g⁻¹) [42], (ii) the same trial as above conducted in Japanese patients ($n=54$) (eGFR 30–75 ml min⁻¹ 1.73 m⁻², ACR >200 mg g⁻¹) [43] and (iii) a phase 2b 8 week trial evaluating the effect of low or high dose atrasentan on ACR and thoracic bioimpedance in CKD patients ($n=45$) on maximal tolerated RAS blockade (eGFR 30–75 ml min⁻¹ 1.73 m⁻², ACR 300–3500 mg g⁻¹) [44]. In addition, a study is in development evaluating the effect of RE-021, a dual ETA antagonist and ARB, on UAER and safety in patients ($n=72$) with primary focal segmental glomerulosclerosis (FSGS) (eGFR >45 ml min⁻¹ 1.73 m², ages 8–50 years) [45]. Finally, a study on the effects of 1 year of bosentan treatment on renal function in patients with scleroderma renal crisis was planned but suspended [46], perhaps based on preliminary data in six patients which did not show a benefit of bosentan over historical controls [47].

Since RAS blockers are standards of care in treating CKD patients, the question arises as to whether ERAs together with RAS blockers confer additional benefit as compared with single agent treatment. Preclinical studies in a model of diabetic nephropathy provide support for the notion that combined ERA and RAS blockade confers greater protection against CKD progression than blockade of either system alone [18]. However, such analysis has not been conducted in human trials. As mentioned earlier, Dhuan and colleagues [36] determined that the acute reduction in proteinuria induced by BQ-123 was greater in patients with chronic proteinuric kidney disease who were taking dual ACEI/ARB therapy as compared with those on ACEI alone, suggesting that ERAs confer an additional benefit in the setting of RAS blockade. However this does not address the question as to whether RAS blockade is efficacious in treating CKD on top of ERA therapy. Since it is highly unlikely that studies will be conducted for the foreseeable future on the effects of ERAs in CKD progression in the absence of RAS blockade, it is probable that ERAs, if ultimately proven to slow CKD progression, will become part of a multidrug approach.

Endothelin antagonists in end-stage kidney disease

ERAs may be of benefit in reducing morbidity and mortality in dialysis patients, although no clinical trial has been conducted. Dialysis patients have elevated plasma ET-1 between 2–10 fold greater than healthy individuals as well as increased platelet ET-1 content and monocyte ET-1 mRNA levels [48–51]. Plasma ET-1 is likely increased in dialysis patients for the same reasons as in CKD patients (inflammation, reduced clearance, hypertension and vascular injury) [52, 53] as well as due to erythropoiesis-stimulating agents [49, 54] and flow through the arteriovenous fistula [55]. In particular, there is a strong correlation between plasma ET-1 and C-reactive protein, atherosclerosis and proximal aorta stiffness in dialysis patients [56–58]. Pre-clinical studies indicate that ET-1 may be important in vascular calcification in the setting of CKD [59], while ETA receptor blockade caused regression of vascular calcification in a model of isolated systolic hypertension [60]. Finally, pulmonary hypertension has been reported to have a high incidence in patients being dialyzed through an arteriovenous fistula [61]. Administration of bosentan to a patient with end-stage renal disease due to scleroderma normalized pulmonary artery pressure and was well tolerated [62]. Based on the above considerations and given the high morbidity and mortality rate in the dialysis population, there should be a strong impetus for examining the potential therapeutic benefit of ERAs in this patient population.

Safety profile of endothelin antagonists

As discussed above, fluid retention is a major clinical concern associated with ERA use. The mechanisms responsible for ERA-induced fluid retention have not been fully elucidated. Blockade of ETB receptors, as could occur with non-selective ETA/B receptor antagonists or high doses of ERAs with relative ETA : B selectivity, would be predicted to cause fluid retention since ETB receptors exert a generally natriuretic effect (reviewed in detail in Kohan *et al.* [1]). However, ERAs with marked ETA selectivity (e.g. sitaxsentan with 6000 : 1 ETA : B selectivity) cause fluid retention in patients and to an apparently comparable degree as that seen with less selective ETA receptor antagonists [63, 64]. How ETA receptor blockers cause fluid retention is uncertain, although blockade of nephron ETA receptors may be involved [1]. The bottom line is that regardless of which ERA is utilized, careful attention must be paid to patient selection (including avoiding patients with severe CKD and/or congestive heart failure who may not tolerate fluid retention), ERA dosing and initiation or increase in diuretic usage.

Sulfonamide-based ERAs have been associated with hepatotoxicity. While this effect was generally mild, sitaxsentan was removed from the market after a few fatal cases [65]. ERAs are teratogenic and therefore contraindicated during pregnancy. In addition, while no peer-reviewed published studies exist, ERAs have been reported in drug company literature to cause testicular toxicity in experimental animals [65]. In this regard, it is encouraging that significant testicular toxicity has not been reported in patients with pulmonary hypertension treated with bosentan or ambrisentan (with the exception of prevention of new digital ulcers in systemic sclerosis, ERAs are only approved for treatment of pulmonary hypertension). In essence, while ERAs appear to have significant therapeutic potential, future clinical trials and ultimately clinical use must be carefully tempered to minimize the side effects of this class of agents.

Conclusions

Abundant pre-clinical data support the notion that blockade of ET-1 actions slows down or prevents progression of chronic kidney disease due to a wide range of underlying causes. In general, the prevailing opinion is that ETA, as opposed to combined ETA/B, receptor antagonists are preferred for treating CKD. Phase 1 and 2 clinical trials have consistently demonstrated that ETA receptor blockade reduces proteinuria in CKD patients. However, as exemplified by the termination of a large phase 3 trial, careful attention must be paid to the side effects of these agents, particularly fluid retention, when treating patients with impaired renal function. Additional clinical trials with ETA receptor antagonists in CKD, including diabetic nephropathy and focal segmental glomerulosclerosis, are ongoing or in the planning stages. There is much optimism for their clinical benefit, however hard renal outcomes analyzing renal function remain to be determined. Finally, consideration needs to be given to conducting trials examining the effects of ETA receptor blockers in dialysis patients.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare D.E.K. and D.M.P. had support from the National Institutes of Health for the submitted work and D.E.K. serves as a consultant for Abbott and Retrophin.

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