Drug Discovery for Overcoming Chronic Kidney Disease (CKD): The Endothelin ET\(_B\) Receptor / Nitric Oxide System Functions as a Protective Factor in CKD

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Abstract. Accelerated cardiovascular disease (CVD) is a frequent complication of renal disease. Chronic kidney disease (CKD) develops hypertension and dyslipidemia, which in turn can contribute to the progression of renal failure. There is general agreement that endothelin-1 (ET-1), which acts through the two subtypes of receptor ET\(_A\) and ET\(_B\), plays important physiological roles in the regulation of normal cardiovascular function and that excessive ET-1 production is linked to CVD and CKD. Although selective ET\(_A\) or nonselective ET\(_A\)/ET\(_B\) receptor antagonisms have been recognized as a potential strategy for treatment of several cardiovascular disease, it remains unclear which of the antagonisms is suitable for the individuals with CKD because upregulation of the nitric oxide (NO) system via ET\(_B\) receptor is responsible for renal function such as natriuresis, diuresis, and glomerular hemodynamics. Our findings clearly indicate that the blockade of ET receptors, in particular ET\(_A\)-receptor antagonism, not only produces a potential renoprotective effect in CKD but also reduces the risk of CVD. In contrast, pharmacological blockade or genetic deficiency of ET\(_B\) receptor seems to aggravate CKD and CVD in several experimental models of rats. Moreover, preliminary evidence in patients with CKD also suggests that both selective ET\(_A\)- and nonselective ET\(_A\)/ET\(_B\)-receptor blockade decreases blood pressure but that selective ET\(_A\) blockade has additional desirable effects on renal hemodynamics. Thus, at least in CKD, these findings support the notion that ET\(_B\) receptor-mediated actions produce a renoprotective effect and that nonselective ET\(_A\)/ET\(_B\)-receptors blockade seem to offer no advantage over selective ET\(_A\) antagonism, and if anything may potentially reduce the benefits.

Keywords: endothelin-1 (ET-1), ET\(_B\) receptor, chronic kidney disease (CKD), nitric oxide (NO), nitric oxide synthase

Introduction

The kidney is a vital organ and its function is decreased by aging. Recent studies have reported that lifestyle-related diseases such as hypertension, dyslipidemia, and diabetes are major risk factors for the development of renal failure (1, 2). In fact, as the elderly population has enlarged, the incidence and prevalence of acute kidney injury (AKI) and chronic kidney disease (CKD) has increased. In addition, there is accumulating evidence that CKD is frequently associated with cardiovascular disease (CVD) because individuals with CKD are more likely to die of CVD than to develop renal dysfunction (3, 4). Levey et al. (5) reported that there was high prevalence of CVD in CKD and that mortality due to CVD was 10 to 30 times higher in dialysis patients than in the general population. These findings suggest that CKD is a risk factor for CVD and that an important strategy for CKD treatments is not only to slow the rate of progression of renal impairment and delay the onset of dialysis in individuals with CKD, but also to improve the cardiovascular risk profile in these
patients. On the other hand, it has been shown that the renin-angiotensin system (6), the sympathetic nervous system (7), inflammation (8, 9), reactive oxygen species (ROS) (10, 11), and nitric oxide (NO) system (12) interact and synergize in the development of CKD and CVD (called the “cardiorenal connection”). Furthermore, recent studies have also demonstrated that blockade of the endothelin (ET) system is one of the potential strategies for CKD because the ET system is closely implicated in kidney diseases (13, 14). In this review, we have summarized our current understanding regarding the role of the ET<sub>β</sub> receptor / NO system as a protective factor in CKD.

**Renoprotective effect of NO system on CKD**

It is widely recognized that abnormal regulation of endothelial function is implicated in the pathophysiology of CKD and that vasoactive substances such as angiotensin II and ET-1 contribute to the progression of CKD. Recent studies have shown that reduced NO production is closely related to the onset and development of CKD (12). The decrease of NO production and/or its bioavailability results from a) the fall of endothelial NO synthase (eNOS) expression and its activity; b) the increase of asymmetric dimethylarginine (ADMA), which is an endogenous competitive inhibitor of eNOS; c) inactivation of NO by ROS; and d) the decrease of active dimer of eNOS based on the degradation of the eNOS cofactor tetrahydrobipterin (BH<sub>4</sub>) (12). Any of these situations would decrease the total NO generated, leading to a reduced NO metabolites (NOx) output. In contrast, there are several reports showing that renal L-arginine synthesis is significantly reduced in patients with advanced CKD (15) and that plasma ADMA levels are correlated to the severity of CKD (16, 17). Indeed, oral supplementation of L-arginine, the substrate of NO, is effective in the renal tissues aggravates the development of the ablation-induced progressive renal failure. On the other hand, Baylis et al. (12) have reported that a deficiency of renal cortical neuronal NOS (nNOS)-derived NO may play a primary role in the progression of CKD. Their observations indicate that the renal cortical nNOS abundance was reduced in several model rats with renal dysfunction including renal mass reduction model rats (12, 27, 28) and that the in vitro NOS activity in the soluble fraction of the renal cortex was decreased in parallel (27–30). They also found that a selective nNOS inhibition greatly accelerated the development of renal damage (31, 32). Taken together, it is reasonable to consider that reduced NO production is implicated in the onset and development of CKD and that both eNOS and nNOS function as a renoprotective factor in CKD.

**Pathophysiological role of ET receptors in CKD**

ET has three family peptides (ET-1, ET-2, and ET-3). ET-1 derived from endothelial cells, smooth muscle cells, and mesangial cells has a much more important role in the regulation of vascular tone than the other isoforms. In fact, many reports on the physiological and pathophysiological profiles of ET-1 indicate that it possesses a number of biological activities that lead to renal disorders. Benigni et al. (33) originally found that ET-1 production was enhanced in renal cortical tissues
from rats with renal mass reduction. Orisio et al. (34) noted that the progression of renal disease after renal mass reduction is closely related to an increase in renal ET-1 gene expression together with an excessive urinary excretion of the corresponding protein. Moreover, in rats with reduction of renal mass, chronic treatment with ET\(_B\)-receptor antagonist reduced the urinary protein excretion, limited glomerular injury, and prevented renal dysfunction (35, 36). In hemodialysis patients with uremia, elevated plasma ET-1 levels have been reported, which correlated with the increase in blood pressure (37). Taken together, it is reasonable to consider that ET-1 action mediated by ET\(_A\) receptors is at least partly contributive to the progression of partial ablation-induced chronic renal failure in rats and that a selective ET\(_B\)-receptor antagonist may be a useful compound for the treatment of human progressive nephropathies.

Physiological and pathophysiological responses to ET-1 in various tissues are mediated by interactions with ET\(_A\)- and ET\(_B\)-receptor subtypes. Both subtypes on vascular smooth muscle cells mediate vasoconstriction, whereas the ET\(_B\)-receptor subtype on endothelial cells mediates vasodilation possibly through the release of NO and prostaglandins (38, 39). Selective ET\(_A\)-receptor antagonists are known to blunt the rise of blood pressure and to attenuate the development of glomerulosclerosis and vascular hypertrophy in chronic renal failure, at least in experimental animals (35, 36). In addition, treatment with nonselective ET\(_A\)/ET\(_B\)-receptor antagonists has also prevented the development of glomerular injury in the same uremic rats (40, 41). In contrast, Shimizu et al. (42) demonstrated that the beneficial effects of an ET\(_A\)-receptor antagonist on proteinuria and renal dysfunction in partial ablation-induced chronic renal failure rats were reversed by concomitant administration with a selective ET\(_B\)-receptor antagonist. Thus, there is general agreement that ET\(_A\) receptor–mediated actions play a crucial role in the development of the ablation-induced chronic renal failure, although some conflicting finding about the effectiveness of an ET\(_A\) receptor has been observed (43). On the other hand, the pathological role of ET\(_B\) receptor–mediated actions in this disease model is not fully elucidated. This led us to evaluate the renal and vascular responses to the partial ablation in the “rescued” ET\(_B\) receptor–deficient \(sl/sl\) (ET\(_B\)\(^{sl/sl}\)) rats (44), the animals that are useful for examining the pathophysiological roles of endothelin ET\(_B\) receptors in renal and vascular tissues. Results clearly indicated that ET\(_B\)\(^{sl/sl}\) rats revealed higher and earlier increases in blood pressure and progression of renal functional insufficiency compared with wild-type (ET\(_B\)\(^{+/+}\)) animals (45, 46). In addition, there were severe glomerular and tubular lesions, enlargement of glomeruli, notable cardiovascular hypertrophy, and increments of renal and aortic ET-1 contents in ET\(_B\)\(^{sl/sl}\) rats. Thus, our findings suggest that ET-1/ET\(_B\) receptor–mediated events in renal tissues and vasculature are protective against the development of the ablation-induced progressive renal failure. This view is consistent with the above pharmacological evidence by Shimizu et al. (42).

Proteinuria is one of criteria in the progression of CKD (47). It is well recognized that reduction of proteinuria is the most important strategy to delay or prevent renal functional loss (47, 48). In the past decade, inhibitors of the renin angiotensin system such as angiotensin-converting enzyme inhibitors and angiotensin II type 1–receptor antagonists have been applied to the patients with chronic proteinuric nephropathies (49). These drugs not only slow down progressive glomerular injury but can even reverse functional injury related to glomerulosclerosis. Although studies in preclinical models of CKD further support the notion that the kidney can recover from glomerular injury, inhibition of renin angiotensin system produces only partial recovery from glomerulosclerosis (50 – 52). Thus, preventive measures and additional therapeutic approaches are urgently needed.

Recent studies have reported that ET-1 exacerbates proteinuria and its hemodynamic effects contribute to glomerular capillary hypertension, an increase in glomerular permeability, and excessive protein filtration (13, 53 – 56). These studies have also indicated that selective ET\(_A\)-receptor antagonists reduce the proteinuria in patients with diabetes and chronic renal failure (13, 54, 56), suggesting that the antiproteinuric effect of ET\(_A\)-receptor antagonism on CKD have a close relation to the changes in glomerular hemodynamics with, potentially, a fall in glomerular capillary perfusion pressure. Taken together, it appears likely that ET antagonists have a renoprotective effect and improve long-term renal outcome.

**ET/NO system in CKD and CVD**

It is well known that vascular endothelial cells excrete various vasoactive substances and that a balance of its production plays an important role in the maintenance of normal endothelial function. A disruption of this balance derived from the endothelial impairment leads to a development of vascular disorders including reduced NO production, ET-1 overproduction, and inflammation. It has been reported that antagonism of the ET system, predominantly with selective ET\(_A\)-receptor antagonists, improves NO-mediated endothelial function (5, 39), suggesting that ET-1, acting via ET\(_A\) receptors, is responsible for the pathogenesis of endothelial dysfunction.
There has been a large number of studies examining the physiological and pathophysiological role of ET-1, its receptors, and NO, but few studies determining the interrelation between ET and the NO system in animal models of CKD in CVD. Other studies (57–59) and our previous studies (60, 61) have shown that ET-1 plays an important role in the pathogenesis of deoxycorticosterone acetate (DOCA)-salt–induced hypertension, on the basis of evidence showing that acute administration of selective ET\(_{\textrm{A}}\)-receptor antagonists or nonselective ET\(_{\textrm{A}}\)/ET\(_{\textrm{B}}\)-receptor antagonists to DOCA-salt rats produces a hypotensive effect and that long-term treatment with these agents suppresses the development of hypertension. Although we have found that chronic treatment with ET\(_{\textrm{A}}\)-selective receptor antagonists improves tissue injuries in DOCA-salt rats (61, 62), the pathological role of ET\(_{\textrm{B}}\) receptor–mediated actions in the DOCA-salt model of hypertension remains controversial. A recent study indicates that nonselective ET\(_{\textrm{A}}\)/ET\(_{\textrm{B}}\) receptor antagonists cause decreases in blood pressure to a degree similar to that found with selective ET\(_{\textrm{A}}\)-receptor antagonists (63). Our results indicated that chronic treatment with a selective ET\(_{\textrm{B}}\)-receptor antagonist to DOCA-salt rats leads to a deterioration in DOCA-salt–induced cardiovascular and renal injuries (62). To develop these results, we used ET\(_{\textrm{B}}\)\(^{+/+}\) rats and examined responses to DOCA-salt treatment. These rats clearly exhibited an exaggerated blood pressure sensitivity to DOCA-salt treatment compared with the sensitivity in wild-type rats (64). In addition, the ET\(_{\textrm{B}}\)\(^{+/+}\) rats had enhanced cardiovascular hypertrophy and worsening of renal dysfunction and tissue damage after the DOCA-salt treatment. These findings lead us to propose that ET\(_{\textrm{B}}\) receptor–mediated actions are protective in the pathogenesis of DOCA-salt–induced hypertension, thereby suggesting that the blockade of this receptor subtype is harmful in such pathological conditions. On the other hand, we have recently developed a new model of hypertensive end-organ damage in rats by the combination of DOCA-salt and a non-selective NOS inhibitor, \(N^\text{o}\)-nitro-L-arginine methyl ester (L-NAME) or \(N^\text{o}\)-nitro-L-arginine (NOARG) (unpublished observation). Two weeks after the start of DOCA-salt treatment, the administration of L-NAME or NOARG for 3 days drastically developed the severe renal dysfunction and tissue injury. In these animals, ET-1 mRNA expression was additionally enhanced in the kidney compared to the rats treated with DOCA-salt alone, and the selective ET\(_{\textrm{A}}\)-receptor antagonist ABT-627 completely prevented renal damage. Thus, although NO prevents a shift toward the development of renal disorders in DOCA-salt rats at 2 weeks, reduced NO production rapidly deteriorates the endothelial function followed by the further increase in blood pressure and the aggravation of renal function. Moreover, ET-1/ET\(_{\textrm{A}}\) receptor–mediated actions are responsible for the increased susceptibility to DOCA-salt–induced hypertension and tissue injuries in the case of reduced NO production.

### ET-1/ET receptors–mediated NOS pathway

ET-1/ET\(_{\textrm{B}}\) receptor signaling is implicated in NO production in the endothelium. It has been shown that ET-1 stimulates eNOS (65–68) and nNOS (69, 70) expression and its activity via predominantly ET\(_{\textrm{B}}\)-receptor activation (65, 67–70). In contrast, Sullivan et al. (71) has recently shown that ET\(_{\textrm{A}}\)-receptor activation upregulates NOS expression and that ET\(_{\textrm{B}}\)-receptor activation controls nNOS and eNOS activity with the renal inner medulla. Thus, although ET-1 increases nNOS and eNOS expression and its activity in several types of cells, the mechanism by which ET-1 regulates these NOS expressions and its activities remains controversial. Our recent observations have indicated that NOx output in the renal tissue by continuous infusion of L-arginine is very markedly lower in ET\(_{\textrm{B}}\)\(^{+/+}\) rats than ET\(_{\textrm{B}}\)\(^{+/−}\) rats (unpublished observation). This reduced converting activity of L-arginine to NO in ET\(_{\textrm{B}}\)\(^{+/+}\) rats was completely recovered by the acute administration of the selective ET\(_{\textrm{A}}\)-receptor antagonist ABT-627. These results suggest that ET-1/ET\(_{\textrm{A}}\) receptor–mediated actions reduce the NO production through the inhibition of NOS activity in ET\(_{\textrm{B}}\)\(^{+/+}\) rats. In addition, we found that both gene and protein expressions of nNOS and eNOS in ET\(_{\textrm{B}}\)\(^{+/+}\) rats were significantly decreased compared with that of ET\(_{\textrm{B}}\)\(^{+/−}\) rats. Thus, ET\(_{\textrm{B}}\)-receptor signaling has a close relation to the NO system, but it remains unclear whether reduced nNOS and eNOS expression in ET\(_{\textrm{B}}\)\(^{+/+}\) rats is dependent on the ET-1/ET\(_{\textrm{A}}\) receptor–mediated actions and/or the ET\(_{\textrm{B}}\) receptor deficiency.

### Conclusion

Nonselective ET\(_{\textrm{A}}\)/ET\(_{\textrm{B}}\) antagonists are currently being used for the treatment of pulmonary hypertension, and selective ET\(_{\textrm{A}}\) antagonists should be approved soon. However, it remains to be clarified whether ET-receptor blockade should be used in CKD and which antagonism is suitable for the individuals with CKD. In this review, we demonstrated that the blockade of ET receptors, in particular ET\(_{\textrm{A}}\)-receptor antagonism, not only produces a potential renoprotective effect in CKD but also reduces the risk of CVD. Preliminary evidence in patients with CKD also suggests that both selective ET\(_{\textrm{A}}\) and nonselective ET\(_{\textrm{A}}\)/ET\(_{\textrm{B}}\)-receptor blockade decreases blood pres-
sure but that selective $\text{ET}_A$ blockade has additional desirable effects on renal hemodynamics. Taken together, at least in CKD, nonselective ET-receptor blockade seems to offer no advantage over selective $\text{ET}_A$ antagonism, and if anything may potentially reduce the benefits. Further studies are needed to clarify the theoretical beneficial effects of an unblocked $\text{ET}_B$ receptor in terms of pathophysiology in CKD.

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References


26 Yamashita C, Tajawa N, Ohkita M, Matsumura Y. Exaggerated


Endothelin ET<sub>B</sub> Receptor/NO System in CKD


68 Rossi NF, Beierwaltes WH. Nitric oxide modulation of ETB receptor induced vasopression release by rat and mouse hypothalamo-neurohypophyseal explants. Am J Physiol Regul Integr Comp. 2006;290:R1208–R1215.


70 Stricklett PK, Hughes AK, Kohan DE. Endothelin-1 stimulates NO production and inhibits cAMP accumulation in rat inner medullary collecting duct through independent pathways. Am J Physiol Renal Physiol. 2006;290:F1315–F1319.