Endothelin receptor antagonists in clinical research — Lessons learned from preclinical and clinical kidney studies

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Abstract

Endothelin receptor antagonists (ETRAs) are approved for the treatment of pulmonary hypertension and scleroderma-related digital ulcers. The efforts to approve this class of drugs for renal indications, however, failed so far. Preclinical studies were promising. Transgenic overexpression of ET-1 or ET-2 in rodents causes chronic renal failure. Blocking the ET system was effective in the treatment of renal failure in rodent models. However, various animal studies indicate that blocking the renal tubular ETAR and ETBR causes water and salt retention partially mediated via the epithelial sodium transporter in tubular cells. ETRAs were successfully tested clinically in renal indications in phase 2 trials for the treatment of diabetic nephropathy. They showed efficacy in terms of reducing albumin excretion on top of guideline based background therapy (RAS blockade). However, these promising results could not be translated to successful phase III trials so far. The spectrum of serious adverse events was similar to other phase III trials using ETRAs. Potential underlying reasons for these failures and options to solve these issues are discussed. In addition preclinical and clinical studies suggest caution when addressing renal patient populations such as patients with hepatorenal syndrome, patients with any type of cystic kidney disease and patients at risk of contrast media induced nephropathy. The lessons learned in renal indications are also important for other potential promising indications of ETRAs like cancer and heart failure.

Introduction

Shortly after the discovery of endothelin-1 (ET-1) in 1988, the entire endothelin system was characterized (Barton and Yanagisawa, 2008). The endothelin (ET) system consists of the three peptides, ET-1, ET-2 and ET-3, their G-protein-coupled receptors endothelin receptor A and

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B (ETAR and ETBR) and the two endothelin-converting enzymes (ECE-1 and ECE-2). The ETAR has a higher affinity for ET-1 and ET-2 than for ET-3, and the ETBR has an equal affinity for all three endothelin isopeptides (Masaki et al., 1994). The ET receptors belong to the family of rhodopsin-like receptors with seven-transmembrane domains coupled to different G proteins and are expressed on the cell surface. However, also nuclear binding sites are described (Hocher et al., 1992). The nonselective ETAR primarily mediates vasoconstriction and thereby is involved in the pathogenesis of hypertension, endothelial dysfunction, inflammation, and fibrosis (Vignon-Zellweger et al., 2012). The peptide-selective ETBR is mainly expressed in vascular endothelial cells, mediates vasodilation via a release of nitric oxide and dilatory prostanooids and inhibits cell proliferation (Vignon-Zellweger et al., 2012). However, ETAR and ETBR can have synergetic or opposing effects depending on cell type, tissue type or physiological situation (Vignon-Zellweger et al., 2012).

The very first ETRAs were peptides and thus were mainly considered research tools. Later Martine Clozel developed an orally available ETA(-) later called bosentan (Clozel et al., 1994). Already shortly after the discovery of ET-1, it became obvious that this new hormone plays a key role in the pathogenesis of renal diseases. However, the efforts to approve ETRAs for renal indications failed so far due to water and salt retention. ETRAs are currently only approved for the treatment of pulmonary hypertension and scleroderma-related digital ulcers (Kohan et al., 2012).

Given the huge prevalence of water and salt retention as most frequently seen side effects, an understanding of renal "ET pathophysiology" with a special focus on safety is key for any clinical development program. The review is thus focused on renal safety aspects of ETRAs. In the first part, however, we also briefly describe experiments showing that a primary -- transgenic -- activation of the ET system causes renal damage on its own followed by a brief summary of preclinical and clinical research with endothelin receptor blockers in diabetic nephropathy.

Lessons learned from rodent models overexpressing ET-1 or ET-2 in the kidney

For the study of the endothelin system several transgenic animal models have been created since its discovery in 1988. A murine animal model transgenic for ET-1, with the transgene expressed predominantly in brain, lung and kidney displayed a strong renal phenotype with the development of renal cysts, interstitial fibrosis of the kidneys, and glomerulosclerosis leading to a progressive decrease in glomerular filtration rate. The observed pathologies are blood pressure independent and developed in spite of only minimally increased plasma concentrations of ET-1 (Hocher et al., 1997). This study further revealed that ET-1 overexpressing mice show an induction of vascular inducible NOS (iNOS) synthase expression in and around renal arteries, possibly causing a local reset of the balance between vascular ET-1 and NO, resulting in no alterations in blood pressure but chronic kidney inflammation characterized by tissue inflammation (increased amount of infiltrating macrophages and CD4+ lymphocytes). The molecular mechanisms leading to this recruitment of immune cells, especially around intrarenal arteries, in ET-1 transgenic mice remain unknown so far (Hocher et al., 2004). The above described pathway is independent of the classical ETB mediated NO release via ETB stimulation of endothelial cells leading to an eNOS activation and subsequently to NO formation (Barton and Yanagisawa, 2008; Masaki et al., 1994). Very elegant work in rodent models with a vascular specific overexpression of ET-1 confirmed the finding of ET-1 induced vascular inflammation. Moreover, using these models, it was shown that ET-1 plays a role in the progression of atherosclerosis and abdominal aortic aneurism formation by decreasing high-density lipoprotein and increasing oxidative stress, inflammatory cell infiltration and matrix metalloproteinase-2 in perivascular fat, vascular wall and atherosclerotic lesions (Amiri et al., 2004; Li et al., 2013).

It is important to realize that not only ET-1 modulates NO synthesis either via the classical ET-1 -> ETB -> eNOS pathway or via ET-1 induced inflammation leading to an upregulation of iNOS (see above). The opposite is also true: NO modulates the synthesis of ET-1. This was demonstrated in elegant studies using either a pharmacological approach to block NO synthesis with l-NAME or a genetic approach (eNOS knockout mice) (Barton et al., 2000; SLOWINSKI et al., 2007; Tharaux et al., 1999). Thus, ET-1 and NO are part of a local paracrine network controlling the synthesis of each other.

ET-2 transgenic rats are characterized by a glomerular expression of ET-2, leading to significantly increased ET-2 tissue concentrations (Hocher et al., 1996a). These animals also developed glomerulosclerosis with significantly increased urinary protein excretion, but yet again the observed pathologies were not caused by an increase in blood pressure. These observations were in line with studies showing that an activated renal paracrine endothelin system is involved in the pathogenesis of glomerulosclerosis (Muñez et al., 1994; Orisio et al., 1993; Roccatello et al., 1994). The lack of hypertension in these models is most likely due to the distinct expression pattern of the transgene and a counter-regulation by other vasoactive systems, like the nitric oxide (NO) system. Findings from studies investigating endothelin transgenic animals suggest that there is a distinct renal effect of transgene overexpression in the kidney. The changes observed are clearly independent of systemic arterial blood pressure, while an isolated renal hypertension and/or increased vascular resistance within the kidney might be present (Liefeldt et al., 1999).

With the help of the ET-1 transgenic mouse model it was demonstrated that overexpressing ET-1 also increases NO bioavailability which counteracts the contractile potency of elevated ET-1 levels and leads to an improvement of endothelium dependent relaxation. Thus, in the presence of an activated ET system, up-regulation of NO production is capable of maintaining vascular tone in a normal range and therefore can prevent the development of hypertension (Quaschning, 2003). The hypothesis that ET transgenic animals do not develop hypertension due to counterregulatory effects by the NO-system was further proven correct by the generation of cross-bred animals of ET transgenic mice and eNOS knockout mice. These animals displayed an even stronger enhancement of blood pressure as compared to the already elevated blood pressure in eNOS knockout mice (Quaschning et al., 2007).

To further elucidate the interaction between the ET and the NO system (Quaschning et al., 2007) a transgenic mouse model carrying a lacZ reporter gene construct under control of the human prepro-ET-1 gene promoter sequence was established. This revealed a close interaction of the renal endothelin and nitric oxide system in a cell-type specific manner, which was especially displayed in renal tubular cells and to a lesser extent in glomerular cells (SLOWINSKI et al., 2007). This study highlighted once more the strong interaction between the ET and the NO system and a major role of ET in renal physiology during adulthood.

Data from transgenic animals show that in adult life the ET system is most important in the renal and cardiovascular system as a chronically activated ET system results in a blood pressure-independent fibrosis of different organs (Von Welby et al., 2009). Transgenic approaches prove that a primary activation of the renal ET system causes kidney damage on its own without other stimuli. The opposite is also true, chronic kidney disease -- classical studies were done in rats with 5/6 nephrectomy -- activates the local renal ET system (Larivière et al., 1997). The interaction of ET-1 and kidney disease thus represents a vicious circle potentially potentiating each other. These observations were a huge stimulus for research focused on developing drugs that suppress the ET system in various fibrotic diseases of the renal and cardiovascular system.

Effects of ET receptor blockers in diabetic nephropathy

ET-1 involvement has been strongly implicated in the pathogenesis and progression of several experimental models of chronic kidney disease such as streptozocin-induced diabetes (Masaki et al., 1994) and type 1 diabetes (Masaki et al., 1994). In the former, ET-1 overexpression was associated with a decreased renal blood flow and an increase in renal resistance, while in the latter, ET-1 overexpression was associated with a decrease in renal blood flow and an increase in renal resistance.

It is likely that ET receptor antagonists could be used to treat diabetic nephropathy by blocking the effects of ET-1 in the kidney. However, more research is needed to determine the optimal dose and duration of treatment for each individual patient.

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disease (CKD), including diabetic nephropathy, glomerulonephritis, hypertensive nephrosclerosis, reduced renal mass and others (Benigni et al., 1998, 2004; Dhaun et al., 2006; Kohan, 2010; Orisio et al., 1993).

More specifically, ET-1 contributes to proteinuria, kidney fibrosis, and renal inflammation in chronic kidney disease. A quite big amount of data suggests that blocking ET receptors improves outcome in a subtype of CKD, diabetic nephropathy. Already in 1998 it was demonstrated that ETAR selective blockade reduced renal injury and improved function in a rat model of diabetes. The observed effects of ETAR antagonism were even stronger than what could be seen with ACE inhibition (Hocher et al., 1998a).

Soon after, Dai etc. conducted a similar study, also using a rat model of diabetes, investigating whether or not the long term treatment with an ETAR antagonist improves outcome in comparison to the established treatment with an angiotensin converting enzyme (ACE) inhibitor. They showed that ETAR antagonism is effective against the typical type I diabetic late complications and regarding renal histological changes, ETAR antagonism is more effective than ACE inhibition (Dai et al., 2000). Until now, numerous other preclinical studies have provided considerable proof of concept data regarding the use of ETR antagonists for the treatment of diabetic nephropathy (Gagliardini et al., 2009; Hocher et al., 2001; Sasser et al., 2007; Watson et al., 2010; Zoya et al., 2011).

One question that prevailed for quite some time was whether combined ETAR and ETBR antagonism as compared to a selective ETAR antagonist would be preferable in treating diabetic nephropathy. Saleh et al. showed that both selective blockade of ETAR and a combined blockade of ETAR and ETBR reduced proteinuria and glomerular permeability and restored glomerular filtration barrier component integrity in a rat model of diabetes. Yet only ETAR selective blockade elicited anti-inflammatory and antifibrotic effects. The authors concluded that selective ETAR antagonists are more likely to be preferred for the treatment of diabetic kidney disease (Saleh et al., 2011). This is in line with the currently available evidence, also suggesting that an ETAR-selective blockade is the more advantageous therapeutic approach (Kohan and Pollock, 2013; Neuhofer and Pittrow, 2009). Interestingly, it was later shown that concomitant ETBR antagonism could potentially even complicate long-term treatment because of the importance of normal ETBR function in avoiding fluid retention (Kohan, 2009).

However, it is important to note that also combined ETA/ETB receptor antagonists such as bosentan are beneficial in experimental diabetic nephropathy (Ding et al., 2003) and improve in patients with diabetes endothelial function and microalbuminuria, key risk factors for disease progression (Rafnsson et al., 2012).

Driven by the positive preclinical results, clinical trials were initiated. These trials reached phases II to III with various ETRAs (atrasentan, avosentan, darusentan, and sitaxsentan) and as observed in preclinical studies reduced proteinuria in patients with CKD (Dhaun et al., 2011; Honing et al., 2000; Kohan et al., 2011; Mann et al., 2010; Wenzel et al., 2009). Despite the success in preclinical studies, none of these ETRAs has been approved by any regulatory agency for the treatment of diabetic nephropathy. On the contrary, alarming observations of long term adverse effects of ETAR antagonism were made in the ASCEND study [A Randomised, Double Blind, Placebo Controlled, Parallel Group Study to Assess the Effect of the Endothelin Receptor Antagonist Avosentan on Time to Doubling of Serum Creatinine, End Stage Renal Disease or Death in Patients With Type 2 Diabetes Mellitus and Diabetic Nephropathy]. This study investigated the effects of avosentan, a predominant ETAR antagonist, on progression of overt diabetic nephropathy in a multicenter, multinational, double-blind, placebo controlled trial. Also the ASCEND study was able to show that avosentan significantly reduces albuminuria when added to guideline-based standard treatment in patients with type 2 diabetes and overt nephropathy, as observed in previous smaller phase 2 clinical studies of shorter duration (Wenzel et al., 2009). However, avosentan also induced a significant fluid overload and congestive heart failure, which leads to a premature termination of the ASCEND study due to an excess in cardiovascular events (Mann et al., 2010). This was a huge drawback for the successful treatment of diabetic nephropathy and CKD in general by ETRAs and led to a strong decline in further studies. Currently, only atrasentan is still being actively studied for the use in patients with CKD (Andress et al., 2012; Kohan et al., 2011). It is currently the hope that the use of diuretics and a rather low dose of atrasentan (minimizing side effect rates) may result in a positive benefit risk balance for the patients.

Based on the fact that blocking ET receptors causes fluid retention, maybe different approaches of modifying the ET system would have avoided this severe adverse effect. Preclinical studies on diabetic rats showed that treatment with a dual inhibitor of both neutral endopeptidase and endothelin-converting enzyme resulted in a decrease of renal matrix protein content as well as protein and albumin excretion independent of blood pressure. The effects were comparable to those of angiotensin converting enzyme inhibition (Thone-Reinke et al., 2004). The benefits of inhibiting both neutral endopeptidase and endothelin converting enzyme result from the different substrates these enzymes process. Inhibiting endothelin converting enzyme blocks the formation of ET-1, which results in positive effects on the progression of diabetic nephropathy. Additionally, by inhibiting the neutral endopeptidase, the degradation of atrial natriuretic peptide (ANP) is blocked. Increasing ANP plasma concentrations while concomitantly inhibiting the ET system should be protective against salt and water retention, as it is known that ANP provides a potent defense mechanism against volume overload in mammals (Antunes-Rodrigues et al., 2004; McGrath et al., 2005).

Safety issues with ET receptor antagonists (ETRAs)

Different ETRAs were tested in various clinical trials involving heart failure, pulmonary arterial hypertension, resistant arterial hypertension, stroke/subarachnoid hemorrhage and several forms of cancer. Results from most of these trials were either negative or neutral, except for pulmonary arterial hypertension and sclerodema-related digital ulcers. Problems with study design, patient selection, drug toxicity, and drug dosing were used to explain or excuse failures (Kohan et al., 2012). Currently, a number of pharmaceutical companies who had developed ETRAs as drug candidates have discontinued clinical trials or further drug development, in some cases due to previously observed serious adverse effects (Mann et al., 2010).

It is interesting that ET antagonism induced testicular toxicity is described in unpublished data of experimental animals and in drug company product literature, but no peer reviewed study has yet addressed this topic (Grass, 2001; Kohan et al., 2012; TRACLEER®).

Water and salt retention

ETRA-induced fluid retention is a very stunning example of how an adverse effect has affected the outcome of clinical trials and may the future of the whole class as a treatment option for CKD. It is noteworthy that all ETRAs used in clinical trials, regardless of receptor isoform specificity, cause fluid retention, including bosentan, darusentan, tezosentan, ambrisentan, sitaxsentan, avosentan, zibotentan and atrasentan (Battistini et al., 2006). The degree of fluid retention is dose-dependent and is aggravated by diseases like congestive heart failure and renal insufficiency. The impact of fluid retention was best illustrated by the failed ASCEND trial in patients with diabetic nephropathy with glomerular filtration rates between 15 and 60 ml/min (i.e., moderate to advanced CKD) (Mann et al., 2010). In retrospect, it turned out that the doses employed in this trial were too high. A previously performed phase II study showed that avosentan displayed antiproteinuric effects at substantially lower doses than those used in the ASCEND trial and caused only modest fluid retention (Wenzel et al., 2009).

The reasons why the higher doses of avosentan caused such significant fluid retention are not completely understood. It is possible that in

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higher doses, as employed in the ASCEND trial, avosentan can also block the ETBR. In vitro studies suggested that avosentan only has a relatively modest selectively for the ETAR (50:1 ETAR:ETBR binding selectivity) (Neuhofer and Pittrow, 2009), so, higher doses of avosentan may also block the natriuretic and diuretic acting ETBR in the kidney. Another possibility is that blockade of ETAR may also cause fluid retention which is dose dependent. Finally, the ASCEND trial involved patients with more advanced kidney disease, who already are more prone to developing fluid retention (Rabelink and Kohan, 2011).

The renal collecting duct (CD) produces and binds more ET-1 than any other region of the kidney and seems to be a key area for ET mediated sodium and water handling. A large number of in vitro studies demonstrated that ET-1 acts in an autocrine fashion to regulate CD function (Kohan, 2011). The physiologic relevance of these findings has been confirmed in vivo by the generation of CD-specific ET-1 and ETR knockouts (KOs) (Ahn et al., 2004; Ge et al., 2005a, 2005b, 2006, 2008). In CD-specific ET-1 KOs, plasma ET-1 levels are not affected, but these animals show a reduced urinary excretion of ET-1. CD ET-1 KO mice are hypertensive but show no differences in body weight, urine volume, creatinine clearance, sodium and potassium excretion, urine and plasma osmolality, plasma aldosterone concentration and renin activity. CD ET-1 KO mice are salt-sensitive and display a reduced sodium excretion during the first three days of a high salt diet. Amiloride and furosemide are able to prevent sodium retention and exacerbation of hypertension. However, they do not reduce blood pressure in CD ET-1 KO mice on a normal sodium diet (Ahn et al., 2004). Plasma vasopressin (AVP) concentrations are substantially reduced in CD ET-1 KO mice, despite all other aspects of water metabolism being similar. However, an increased renal sensitivity to the effects of AVP can be observed, suggesting that ET-1 acts as a physiological autocrine regulator of AVP action in the collecting duct (Ge et al., 2005a). A CD-specific knockout of the ETAR has no effect on blood pressure or urinary sodium excretion in mice, independently of salt intake (Ge et al., 2005b). On the contrary, collecting duct ETBR knockout mice have salt-sensitive hypertension (Ge et al., 2006). However, collecting duct knockout of both ETAR and ETBR causes greater hypertension and sodium retention than in mice with only ETBR disruption (Ge et al., 2008), yet again underlining that both ET receptors are involved in salt and water handling.

It is also known that the ET-1 gene is an early response gene of aldosterone. Because aldosterone and ET-1 have opposing actions on sodium reabsorption in the kidney, it was hypothesized that stimulation of ET-1 by aldosterone acts as a negative feedback mechanism localized in the CD. Aldosterone-mediated sodium reabsorption is, at least in part, caused by stimulating the epithelial Na channel (ENaC). In contrast, ET-1 increases sodium and water excretion through its binding to receptors in the CD. Part of this effect may be due to decreased Na/K-ATPase activity (Zeidel et al., 1989). However, current literature suggests an inhibitory effect of ET-1 on the epithelial Na channel (ENaC). Studies in cultured CD (Pavlov et al., 2010), distal nephron A6 cells (Gallego and Ling, 1996), and 3T3 cells stably expressing ENaC (Gilmore et al., 2001) showed that ET-1 can reduce both channel open probability and cell surface number of this sodium channel (Kohan, 2011). Experiments in isolated split open rat cortical CD showed that ET-1 dynamically regulates ENaC open probability through a signaling pathway including src kinases and MAPK1/2 (Bugaj et al., 2008). Other signaling pathways have also been implicated in ET-1 dependent regulation of distal nephron sodium transport likely mediated by ENaC. There is strong experimental evidence that NO signaling plays a role in ET-1 regulation of distal nephron sodium handling (Schneider et al., 2008). Similarly, modification of medullary blood flow and an increase in production of locally derived signaling factors in response to ET- might as well play a role in ET-1 mediated regulation of collecting duct sodium transport (Brodsky et al., 2000; Escalante et al., 2002; Evans et al., 2001). The relative physiological importance and the relationship between these possible mechanisms of ET-1 control of ENaC remain unclear (Bugaj et al., 2008). In a very recent paper Lynch et al. further unraveled how endothelin acts on ENaC. As seen previously, the presence of ET-1 significantly inhibited ENaC mediated sodium reabsorption. Blockade of either ETAR or ETBR restored this reabsorption to control rates. However, only ETAR blockade restored a benznil-sensitive component of sodium reabsorption. By the addition of various specific channel blockers the authors were able to demonstrate that ET-1 inhibits sodium reabsorption in the cortical CD through both ETAR and ETBR mediated pathways. ETAR blockade restored a benzamil-sensitive component of Na reabsorption, which presumably was ENaC, whereas ETBR blockade restored a benznil-insensitive Na reabsorptive mechanism. The authors concluded that sodium reabsorption is mediated by ENaC in the cortical CD and outer medullary CD and also by an ENaC independent mechanism in the cortical CD. Additionally, ET-1 inhibits sodium reabsorption through both ETAR and ETBR mediated pathways (Jeanette Lynch et al., 2013). Analyzing patients getting adverse events in the clinical trials using endothelin receptor blockers, it became obvious that a main risk factor for fluid retention in humans seems to be the degree of kidney and heart functions rather than selectivity of the ETR antagonist (Benigni et al., 2004; Dhaun et al., 2006; Kohan, 2010; Kohan et al., 2012). In this context, it is also puzzling that water and salt retention is not a major issue in patients with pulmonary hypertension treated with endothelin receptor antagonists. The underlying mechanisms are poorly understood.

**Acute renal failure**

Until now, the pathogenesis of radiocontrast nephrotoxicity is not completely understood, but it has been proposed that an imbalance between vasodilation and vasoconstriction is causative for renal medullary ischemia (Heyman et al., 1991). In animal models of radiocontrast nephrotoxicity it was demonstrated that there is a significant increase in both plasma and urinary endothelin levels, during and after intravenous radiocontrast infusion (Margulies et al., 1991). Furthermore, it was shown that administration of ETARs prevents renal vasoconstriction in animal models of radiocontrast nephrotoxicity (Brooks and DePalma, 1996; Cantley et al., 1993). However, these observations did not translate into a treatment option for radiocontrast nephrotoxicity in humans. In a multicenter, prospective, randomized study, investigating the use of a combined ETAR/ETBR antagonist in patients with CKD undergoing radiocontrast assisted cardiac angiography, it was shown that ER antagonism and intravenous hydration exacerbate radiocontrast nephrotoxicity compared with hydration alone (Wang et al., 2000). However, it is noteworthy that this phase 3 trial was not based on phase 2 studies to establish the right dose. The authors and the responsible company took a huge risk of failure by not having done a phase 2 program to define a potentially better dose for clinical use.

**Polycystic kidney disease**

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic hereditary kidney disease in humans, which is seen in about 1 in 1000 live births. This disease accounts for up to 10% of all patients requiring renal replacement therapy (Hocher et al., 1998b). An initial observation that ET-1 concentrations are markedly increased in ADPKD patients with severe renal failure led to the hypothesis that ET-1 plays a pathogenic role in ADPKD (Hocher et al., 1998b). This hypothesis was supported by the finding that administration of ETARs prevented renal vasoconstriction in animal models of radiocontrast nephrotoxicity (Brooks and DePalma, 1996; Cantley et al., 1993). However, these observations did not translate into a treatment option for radiocontrast nephrotoxicity in humans. In a multicenter, prospective, randomized study, investigating the use of a combined ETAR/ETBR antagonist in patients with CKD undergoing radiocontrast assisted cardiac angiography, it was shown that ER antagonism and intravenous hydration exacerbate radiocontrast nephrotoxicity compared with hydration alone (Wang et al., 2000). However, it is noteworthy that this phase 3 trial was not based on phase 2 studies to establish the right dose. The authors and the responsible company took a huge risk of failure by not having done a phase 2 program to define a potentially better dose for clinical use.
Brochu et al., 1999; Hocher et al., 2001; Orth et al., 1999). Regardless of the negative results of ETRAs in ADPKD, other groups continued to investigate ETAR or ETBR blockade in animal models of ADPKD, reasoned by an availability of new, highly selective, orally active nonpeptide ETRAs. Because ETBR had been shown to mediate tubular cell proliferation in vitro, it was hypothesized that ETBR blockade would decrease tubular cell proliferation (Chang et al., 2007). Unexpectedly, treatment with an ETBR antagonist leads to an exacerbation of the renal cystic phenotype with a reduction in urine volume and sodium excretion and increases in urine osmolarity and renal CAMP and ET-1 concentrations. Simultaneous ETAR blockade reversed the severe changes that were seen after sole ETBR blockade. ETAR blockade alone resulted in a significant increase in tubular cell proliferation but did not alter the cystic phenotype (Chang et al., 2007), which is consistent with previous reports (Hocher et al., 2003).

Taken together, studies utilizing selective or non-selective ETAR and ETBR antagonists to ameliorate cystic disease progression in rodent PKD models have proven disappointing and do not support further extension into clinical trials. The results of these studies show that a critical balance between ETAR and ETBR action in the cystic kidney appears to be necessary to maintain kidney structure and function. Current evidence suggests that ET-1 and its receptors act as major modifying genes for renal disease progression in ADPKD but different approaches are needed to translate these findings into clinical practice (Chang and Ong, 2011).

Renovascular hypertension

In two kidney–one clip renovascular hypertension (2K1C), blood flow is reduced in the clipped kidney leading to ischemia, whereas in the non-clipped kidney shear stress and blood pressure are increased. Based on previous reports, which showed that parts of the paracrine renal ET system (tissue ET-1 and ETAR density) are upregulated in the clipped kidney in the late phase of renovascular hypertension in rats with 2K1C renovascular hypertension (Diekmann et al., 2000), a study was initiated investigating potential positive effects of endothelin antagonism on reducing the progression of kidney fibrosis. Long term blockade of the activated endothelin system in the clipped kidney of rats with renovascular hypertension using an ETAR antagonist did not improve symptoms but lead to a fibrotic atrophy of the clipped kidney. The effects of ETAR antagonists on the non-clipped kidney were less pronounced. Neither blood pressure nor plasma renin activity was significantly altered by ETAR blockade treatment. The authors suggested that the ETAR blockade might reduce perfusion pressure in the clipped kidney to a critical extent, thus causing ischemia induced fibrotic atrophy, similar to what is observed when blocking the renin angiotensin system with concomitant renal artery stenosis (Hocher et al., 2000).

Anemia

Anemia is a strong and independent risk factor for morbidity and mortality in a number of chronic diseases (Akizawa et al., 2008; Knight et al., 2004). One of the few authority approved applications of ET antagonism is pulmonary hypertension (Dingemanse and van Giersbergen, 2004). In a quite recent study it was shown that hemoglobin levels closely parallel survival in patients with pulmonary hypertension. The authors point out that modification of anemia in this disorder could alter the clinical course and call for further research in this area (Krasuski et al., 2011). However, in the above mentioned study no effect of bosentan, a dual ETRA, on hemoglobin status could be seen. It is noteworthy though that the total number of patients included in this study was only 145 patients, of whom only 17% were treated with bosentan. It is very likely that the number of patients receiving bosentan was too low to observe any difference in occurrence of anemia. Actually, anemia is a very common adverse effect of ETRAs, seen in various clinical trials of several different compounds (Dingemanse and van Giersbergen, 2004; Ma et al., 2012; Pulido et al., 2013; Vergouwen et al., 2012). Current evidence suggests that the higher risk of anemia during treatment with an ET antagonist might not just be an association but causally linked to ET antagonism. In a study by Föller et al. it was shown in vitro and in vivo that a stimulation of the ETBR inhibits suicidal erythrocyte death, which is characterized by phosphatidylserine exposure at the erythrocyte surface and trigger by increases in cytosolic calcium via glucose withdrawal. ET1 and sarafotoxin 6c, an agonist of ETBR, did not significantly modify cytosolic calcium activity or phosphatidylserine exposure in isotonic, glucose-containing, extracellular fluid. Both ET1 and sarafotoxin 6c however, significantly blunted the effect of glucose withdrawal on phosphatidylserine exposure. The study also evaluated the in vivo significance of the findings. To this end rescued ETBR knockout and wild-type mice were used. The number of phosphatidylserine-exposing erythrocytes, reticulocytes and spleen size was significantly larger in ETBR-KO mice than in wild-type mice. The ETBR-KO erythrocytes were more susceptible to the apoptotic effect of oxidative stress and more rapidly cleared from circulating blood than ETBR-KO erythrocytes (Föller et al., 2010).

These results coming from animal studies could be of major clinical significance and should stimulate future clinical studies to assess whether anemia during treatment with currently approved ET antagonists is due to suicidal erythrocyte death.

Pregnancy

Results from the different transgenic and knockout models show that the ET system plays an important role in embryonic development. Homozygous ET-1 KO mice display severe craniofacial abnormalities. These mice die soon after birth from asphyxia, because they cannot breathe normally due to a missing jaw (Kurihara et al., 1994). Furthermore they display malformations of the cardiovascular system (Kurihara et al., 1995a), thymus and thyroid (Kurihara et al., 1995b). No abnormalities in other organs such as the lung, kidney and central nervous system can be observed. Similar craniofacial and cardiovascular malformations are seen in mice with disruption of the ETAR gene (Clouthier et al., 1998). The important role of ET-1 in skeletal development during intrauterine life was confirmed by using prepro-ET-1-lacZ-transgenic mice (Słowinski et al., 2007). The examination of expression patterns of ETARs and ET-1 suggests that ET-1/ETAR interaction is essential in cranial bone and connective tissue formation as well as in the development of the heart and its outflow tract (Clouthier et al., 1998). In the case of ETBR knockouts, a developmental failure of epidermal melanocytes leads to a colorless spotted skin and an enteric neuron defect that result in a megacolon, because the gastrointestinal tract cannot undergo peristaltic movements anymore (Hosoda et al., 1994; Shin et al., 1999).

Succeeding studies in which pharmacological blockade of the receptor was tested at different timepoints throughout gestation identified that blocking ETAR in early to mid-gestation resulted in phenotypical birth defects similar to those seen in the knockout model (Taniguchi and Muramatsu, 2003). All ETAR antagonists currently on the market list pregnancy as a contraindication and the FDA recommends pregnancy tests at frequent intervals while on treatment (Kingman et al., 2009).

It is known that endothelin plays an important role in one of the most common pregnancy complications, preeclampsia (George et al., 2012). Antagonism of the ET system has been proven beneficial in numerous animal models of gestational hypertension, and it remains a putative target for pharmacological intervention in this disease (George et al., 2012). However, given the current state of knowledge, this seems unlikely. The problem of teratogenicity might be manageable, as some studies demonstrated that the administration of a selective ETAR antagonist only in late gestation has no teratogenic effect in either mice or rats (Olgun et al., 2008; Thaete et al., 2001). But regarding the potential

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of ET antagonism to cause salt and water retention, it appears not beneficial to use such a pharmacological approach as fluid retention is a pathognomonic feature of preeclampsia (Davidson, 1997).

Hepatorenal syndrome

As a pathophysiological consequence, patients with impaired liver function often develop renal failure. Depending on time course and severity, we distinguish between type 1 and type 2 hepatorenal syndrome (HRS). Renal vasoconstriction is a key factor in the development of HRS and may be secondary to increased activities of ET-1, a potent renal vasoconstrictor. In rats, experimental liver cirrhosis by CCl₄ (carbon tetrachloride) causes an up-regulation of the ETBR in the renal medulla. Blocking this receptor with a non-selective ETAR/ETBR antagonist causes water and salt retention in these rats with CCl₄-induced liver cirrhosis (Hochez et al., 1996b). Despite these preclinical data, a clinical study was initiated to test exactly the same hypothesis in humans. This small, but well done study demonstrated, in line with the earlier preclinical data, that endothelin receptor blockade potentially can cause a deterioration in renal function in patients with liver cirrhosis and HRS (Wong et al., 2008). Thus, caution should be taken in future studies using endothelin receptor antagonists in patients with liver cirrhosis.

Conclusions

ETRAs are a very promising class of drugs for the treatment of various cardiovascular and renal diseases. Preclinical work also suggests the use of these drugs in various types of cancer such as prostate cancer. However, the mode of action of ETRAs causes renal side effects in general. All clinical development programs will face the fact that both blocking the ETAR and also the ETBR causes water and salt retention. Thus careful patient selection is essential — patients with a high edema and/or water and salt retention risk should be excluded. In addition, measures to monitor and treat these side effects are key parts of any future clinical study. These class-related side effects are manageable and should not block the clinical development of ETRAs in the future. Besides these general points to consider, there are subgroups of patients with kidney diseases that should not receive ETRAs based on our current knowledge. Data indicate that kidney function may be worsened by blocking the ET system in:

- Chronic liver failure
- Any type of cystic kidney disease
- Patients at risk of AKI
- Patients with renovascular hypertension

In addition, recent experimental work showed that eNOS knockout mice develop diastolic dysfunction, which could be rescued by ET-1 transgenic overexpression in the heart. This study furthermore suggested that cardiac ET-1 overexpression in case of eNOS deficiency interfered with the regulation of proteins playing a role in oxidative stress, myocyte contractility, and energy metabolism (Vignon-Zellweger et al., 2011). Thus, in conditions of diastolic heart failure with impaired endothelial function it might not be a good idea to block the ET system at all.

Moreover, there is a smart way to overcome the intrinsic side effect of water and salt retention caused by pure endothelin receptor antagonists that would merit more attention by clinical researchers in the future: combined endothelin converting enzyme and neutral endopeptidase inhibitors (Kalk et al., 2011; Sharkovska et al., 2011; Thöne-Reinke et al., 2004; Wengenmayer et al., 2011). This new class of drugs may have all the beneficial effects of pure endothelin receptor blockade and may solve the problem of water and salt retention at the same time by raising plasma levels of natriuretic peptides such as ANP (Kalk et al., 2011; Sharkovska et al., 2011; Thöne-Reinke et al., 2004; Wengenmayer et al., 2011). However, clinical data with the new class of drugs are urgently needed to allow firm statements. A head to head comparison of NEP/ECE inhibitors with ET receptor blocking agents in combination with diuretics would generate important clinical information and guidance for future clinical use.

Conflict of interest statement

None of the authors have any conflict of interest with regard to the review topic.

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