REVIEW

Renoprotective Effects of ET$_A$ Receptor Antagonists Therapy in Experimental Non-Diabetic Chronic Kidney Disease: Is There Still Hope for the Future?

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Summary

Chronic kidney disease (CKD) is a life-threatening disease arising as a frequent complication of diabetes, obesity and hypertension. Since it is typically undetected for long periods, it often progresses to end-stage renal disease. CKD is characterized by the development of progressive glomerulosclerosis, interstitial fibrosis and tubular atrophy along with a decreased glomerular filtration rate. This is associated with podocyte injury and a progressive rise in proteinuria. As endothelin-1 (ET-1) through the activation of endothelin receptor type A (ET$_A$) promotes renal cell injury, inflammation, and fibrosis which finally lead to proteinuria, it is not surprising that ET$_A$ receptors antagonists have been proven to have beneficial renoprotective effects in both experimental and clinical studies in diabetic and non-diabetic CKD. Unfortunately, fluid retention encountered in large clinical trials in diabetic CKD led to the termination of these studies. Therefore, several advances, including the synthesis of new antagonists with enhanced pharmacological activity, the use of lower doses of ET antagonists, the addition of diuretics, plus simply searching for distinct pathological states to be treated, are promising targets for future experimental studies. In support of these approaches, our group demonstrated in adult subtotally nephrectomized Ren-2 transgenic rats that the addition of a diuretic on top of renin-angiotensin and ET$_A$ blockade led to a further decrease of proteinuria. This effect was independent of blood pressure which was normalized in all treated groups. Recent data in non-diabetic CKD, therefore, indicate a new potential for ET$_A$ antagonists, at least under certain pathological conditions.

Introduction

Chronic kidney disease (CKD) is a life-threatening disease arising as a frequent complication of diabetes, obesity and hypertension. Since it is typically undetected for a long time, it often progresses to end-stage renal disease. Prevalence of CKD is sharply increasing in recent decades due to the ageing of the world population: affecting more than 10 % of the adult population. CKD is characterized by the development of progressive glomerulosclerosis, interstitial fibrosis and tubular atrophy along with a decreased glomerular filtration rate. This is associated with podocyte injury and a progressive rise in proteinuria.

Angiotensin converting enzyme inhibitors (ACEi) and angiotensin type 1 receptor blockers (ARB) are used as the "gold standard" in the treatment of CKD due to their antihypertensive and renoprotective effects. However, the renin-angiotensin system (RAS) blockade only partially slows down the progression of CKD to

end-stage renal disease. Although other additional strategies on top of RAS blockade are used for the management of this disease, the tremendous interest in new therapeutics is apparent. As endothelin-1 (ET-1) leads through the activation of ET_A receptors to renal cell injury, inflammation, fibrosis and finally to proteinuria, it is not surprising that ET_A receptor blockers were proven to have beneficial renoprotective effects in both experimental and clinical studies (Barton 2008, Barton and Yanagisawa 2008, Kohan and Pollock 2013, Kohan and Barton 2014, Komers and Plotkin 2016, Czopek et al. 2016). However, due to the presence of edema encountered in clinical trials with ET_A receptor blockade, ASCEND and RADAR clinical trials have been prematurely terminated. Although the SONAR trial attempted to overcome these complications by using a lower dose of atrasentan, the trial was also terminated before its planned conclusion. Thus, only the DUET trial evaluating sparsentan – the dual antagonist of AT_1 and ET_A receptors (comprising moieties of irbesartan and atrasentan in one molecule) – in patients with primary focal segmental glomerulosclerosis (Komers et al. 2017) (DUET trial NCT01613118), ambrisentan in patients with sickle cell nephropathy (NCT02712346 trial), and the ZEBRA trial (NCT02047708) evaluating highly selective ET_A antagonist zibotentan in scleroderma patients provide optimistic indications to this area of research at present.

This review focuses on the experimental data obtained in non-diabetic chronic kidney disease with a focus on selective versus non-selective ET receptor antagonists in hypertension and associated end-organ damage resulting in CKD. We shall also examine the use of diuretics in CKD.

Endothelin-1 and its receptors

ET-1 is the best known and predominant isoform among the family of endothelins (ET-1, ET-2, and ET-3) (Yanagisawa et al. 1988). It is a more potent and longer lasting vasoconstrictor in humans than angiotensin II (Ang II). ET-1 exerts its action through two types of G-protein coupled receptors, ET_A and ET_B. While ET_A receptors located on vascular smooth muscle cells mediate vasoconstriction and cell proliferation, the effects of ET_B receptors located mainly on endothelial cells include vasodilation through the release of nitric oxide and prostacyclin (Hirata et al. 1993), ET-1 clearance and inhibition of sodium reabsorption in the renal

collecting duct, thus contributing to the regulation of sodium and water homeostasis (for review see Kohan 2013, Maguire and Davenport 2014, Boesen 2015, Davenport et al. 2016). Moreover, it must be pointed out that there is also a diurnal regulation of kidney function contributing to the proper water and electrolyte homeostasis (Zhang and Pollock 2018). ET-1 stimulation of ET_A receptors leads to proliferation and fibrosis, while the ET_B receptors mediate opposite effects. The interrelation of vasoconstrictor pathways, i.e. the activation of ET-1 by Ang II and vice versa, has been known for more than 20 years (Kohno et al. 1992, Kawaguchi et al. 1990, Barton et al. 1997). Recently, the interrelationship between the vasodilator pathways of renin-angiotensin and endothelin systems, namely the crosstalk between Ang-(1-7) and ET-1 through the Mas receptor and ET_B receptor, respectively, has been described (Hood et al. 2016).

Non-selective versus selective ET receptor antagonists in experimental hypertension

There has been considerable debate among researchers and clinicians on the benefits of the non-selective versus selective endothelin receptor blockade which arose from the controversial results obtained in experimental studies. While some authors reported antihypertensive or nephroprotective effects of selective ET_A receptor blockade in various hypertensive models – Sabra salt-sensitive rats (Rothermund et al. 2003), DOCA-salt hypertensive rats (Allcock et al. 1998, Matsumura et al. 1999), salt-loaded stroke-prone SHR (Okada et al. 1995), or adult Dahl salt-sensitive rats (Barton et al. 1998, Okada et al. 2000, Zicha et al. 2012), no protective effects of ET_A blockade were found in young and adult Ren-2 transgenic rats harboring additional mouse renin gene (TGR) (Rossi et al. 2000, Rothermund et al. 2003), inducible TGR (CYP1A1) rats (Vaňourková et al. 2009), and young Dahl salt-sensitive rats (Zicha et al. 2012). On the other hand, our group has demonstrated a mild hypotensive effect of ET_A blockade with atrasentan in both young and adult TGR which was associated with cardio- and renoprotection (Vaněčková et al. 2005, Opočenský et al. 2006, Vaněčková et al. 2006, Vernerová et al. 2008). In young homozygous TGR rats, the antiproteinuric effect of atrasentan was found despite the persistence of severe hypertension (Vaněčková et al. 2006).

There is no doubt about the efficacy of bosentan

in pulmonary hypertension (Clozel *et al.* 2006). Concerning other hypertensive models, non-selective ET receptor blockade was found to be organoprotective in homozygous TGR (Dvořák *et al.* 2004, Opočenský *et al.* 2004), double transgenic rats (Muller *et al.* 2000) and rats with diabetic nephropathy (Ding *et al.* 2003). Antihypertensive effects of non-selective ET receptor blockade were demonstrated in DOCA-salt hypertensive rats (Schiffrin *et al.* 1996), double transgenic rats (Muller *et al.* 2000), young TGR (Rossi *et al.* 2000) and 2K1C Goldblatt hypertensive rats (Li *et al.* 1996). However, our group repeatedly demonstrated the absence of hypotensive effect of bosentan in both heterozygous and homozygous TGR on either normal or high-salt diets (Dvořák *et al.* 2004, Vaněčková *et al.* 2005, Vaněčková *et al.* 2006, Opočenský *et al.* 2006, Vernerová *et al.* 2008).

Chronic kidney disease

Endothelin 1 is known to be associated with the development and progression of chronic kidney disease and associated cardiovascular complications. Thus, the beneficial antiproteinuric effects of selective endothelin receptor A blockade were not surprising in patients with chronic renal disease (Dhaun *et al.* 2011, Gagliardini *et al.* 2011, Kohan and Barton 2014), especially in those with diabetic CKD. However, adverse side effects (edema) led to the termination of three large clinical trials with ET$_A$ blockade (ASCEND, RADAR, SONAR) in patients with diabetic nephropathy (www.clinicaltrial.gov, Nrs. NCT00120328, NCT01356849, NCT01858532, respectively). On the other hand, positive results of the dual blockade with sparsentan in patients with focal segmental glomerulosclerosis in the DUET trial (Komers *et al.* 2017, Komers 2017), showing better antiproteinuric effects of sparsentan as compared with AT$_1$ receptor blocker irbesartan, support further experimental testing. In addition, other approaches including the synthesis of new antagonists with enhanced pharmacological activity, such as macitentan (Bolli *et al.* 2012), the use of lower doses of ET antagonists (Baltatu *et al.* 2014), or the addition of diuretics (Jin *et al.* 2014, Morales *et al.* 2015, Fuwa *et al.* 2016, Vaněčková *et al.* unpublished results, see below) could be promising. Another important consideration comes from the field of genetics – the finding of rs9349379, a common SNP in PHACTR1 gene, as a putative causal variant of five common diseases (coronary artery disease, migraine,

cervical artery dissection, fibromuscular dysplasia, and hypertension), and its distal regulatory effect on endothelin-1 expression (Gupta *et al.* 2017). While the proposed mechanism(s) of action of the first four diseases is mediated through ET$_A$ receptors and is associated with an increased risk of coronary artery disease and a decreased risk of the other three diseases, decreased risk of hypertension is connected with the hypotensive actions of ET$_B$ receptors on endothelial cells of large arteries and renal collecting ducts and is connected with lower risk of hypertension. Perhaps, this might be one of the possible explanations for conflicting results in different CKD models.

Proteinuria and podocytes

Proteinuria is an indicator of deteriorated kidney function which arises from the defect of the filtration barrier in the kidney, affecting mainly podocytes. The reduction of proteinuria incidence is an important goal in the treatment of kidney disease. Podocyte injury is associated with many disease conditions, such as, focal segmental glomerulosclerosis, minimal change disease, and membranous nephropathy. Although the primary cause may differ, the derangement of the actin cytoskeleton leading to the loss of functional integrity of the glomerular filtration barrier and to podocyte detachment is a common feature of these diseases. Considerable attention has been given to the remodeling of podocytes in both diabetic and non-diabetic nephropathies (Pavenstadt *et al.* 2003, Hayden *et al.* 2005, Fligny *et al.* 2011, Raij *et al.* 2016). The remodeling of podocytes consists of cellular swelling, effacement of foot processes, vacuole formation, fusion of foot processes, and finally their detachment from the glomerular basement membrane (Mallipattu and He 2016). Different therapeutic strategies, including the use of RAS blockers, glucocorticoids or calcineurin inhibitors, were partly effective in alleviating podocyte damage. An important role in this deleterious process also involves ET-1, through the activation of ET$_A$ receptors (Barton and Tharaux 2012) and also ET$_B$ receptors (Lenoir *et al.* 2014). Thus, the restoration of proper podocyte functioning is the aim of various pharmacological interventions, including ET receptor blockade. Important information on the role of podocytes comes from older studies performed in Dahl hypertensive rats (Nagase *et al.* 2006), aged Wistar rats (Ortmann *et al.* 2004), or DOCA-salt hypertensive rats (Kretzler *et al.*

1994) which demonstrated that podocyte injury precedes the development of glomerulosclerosis and established hypertension. Our study in TGR rats (Opočenský *et al.* 2006) also supported this concept showing that glomerular podocyte injury was substantially reduced with ET_A receptor antagonist atrasentan (independent of severe hypertension) and this injury strongly correlated with the survival of animals suggesting the major role of ET_A receptor in this process. In addition, Lenoir *et al.* (2014) demonstrated in diabetic transgenic podocyte-specific ET_A and ET_B knockout mice that not only ET_A but also ET_B receptors may be involved in the deleterious effects of ET-1 on podocytes. Moreover, the study by Garsen *et al.* (2016) demonstrated that ET-1 signaling through the increase in heparanase in podocytes leads to the disruption of glomerular glycocalyx which consequently resulted in proteinuria and renal damage (Garsen *et al.* 2016). ET_A receptors are responsible for this process since atrasentan treatment through the decrease in heparanase expression induced the increase in glycocalyx thickness and led to the reduction of albuminuria (Boels *et al.* 2016).

Endothelin antagonists in experimental non-diabetic CKD

The positive effects of ET receptor blockade have been demonstrated shortly after ET-1 discovery in experimental studies of CKD (Benigni *et al.* 1991, Benigni *et al.* 1993, Benigni *et al.* 1996, Brochu *et al.* 1999). These beneficial effects were suggested to be associated with the intervention to the activated endothelin system following kidney ablation or renovascular hypertension (Orisio *et al.* 1993, Diekmann *et al.* 2000). Previous studies have shown that urinary ET-1 excretion correlates with kidney damage and proteinuria (Orisio *et al.* 1993). Recent study in TGR rats showed that the rise in renal ET-1 concentration in the early phase after 5/6 nephrectomy was 50-fold (Sedláková *et al.* 2017). In fact, urinary ET-1 excretion, which reflects renal ET production, is increased not only in renovascular hypertension or nephrectomized animals but in all forms of CKD including diabetes, hypertension, glomerulonephritis, and others (Kohan and Barton 2014). Urinary ET-1 excretion increased with the deterioration of kidney function also in patients with CKD (Kohan and Pollock 2012, Dhaun *et al.* 2009) and could potentially serve as a marker of renal damage. In contrast, plasma ET-1 levels are considered as an unreliable marker due to

the technical problems encountered with its measurement, rapid receptor-mediated uptake, and especially due to its paracrine action. Recently, in patients with chronic kidney disease the plasma concentrations of stable precursors of ET-1 synthesis (C-terminal pro-ET-1 peptide and endothelin-like domain peptide) were demonstrated to be good markers of kidney injury which negatively correlated with glomerular filtration rate (Dhaun *et al.* 2015). In addition, ET_A receptor blockade increased their levels reflecting the upregulation of ET-1 gene, which could be responsible for fluid retention encountered with ET_A receptor blockade. However, more clinical and experimental studies are needed to support this finding.

Since the role of the endothelin system in chronic kidney disease was exhaustively summarized by Kohan and others (Kohan 2013, Kohan and Barton 2014, Kohan and Pollock 2014, Culshaw *et al.* 2016), the present review will focus only on the recent experimental studies in this field.

Baltatu *et al.* (2014) demonstrated in double transgenic rats (harboring human renin and the angiotensinogen gene) that the treatment with a low doses of avosentan in combination with low dose of valsartan for five weeks significantly decreased albuminuria and renal damage with no signs of fluid retention. Our results in TGR rats with ablation nephropathy also supported the positive antiproteinuric effects of ET_A blockade using atrasentan combined with maximal RAS blockade (Čertíková Chábová *et al.* 2014, Vaněčková *et al.* unpublished data, see below), although for the manifestation of its positive effects, the treatment must be very long (approximately one year) (Čertíková Chábová *et al.* 2014). When the duration of treatment was only 20 weeks, the positive effects were not observed (Vaněčková *et al.* 2012). This is consistent with a study in 5/6 nephrectomized female Sprague Dawley rats in which a three-month treatment with atrasentan in combination with enalapril (ACEi) and paricalcitol (vitamin D activator) had no additional renoprotective effects as compared to the treatment with enalapril alone (Ritter *et al.* 2014). Unfortunately, beneficial effects were also not demonstrated in TGR rats in which the long-term treatment with ET_A blockade on top of RAS blockade was delayed to the phase of established hypertension and CKD (Sedláková *et al.* 2017). On the other hand, in a swine model of renovascular disease, the postponed treatment with ET_A receptor blockade (but not ET_B blockade) was successful in attenuation of the

progression of renal inflammation and fibrosis in the stenotic kidney, which was associated with improved hemodynamic parameters and decreased albuminuria (Chade *et al.* 2014). In addition, the same group demonstrated that non-selective ET antagonist macitentan had anti-inflammatory effects, improved hemodynamics of the stenotic kidney and reduced albuminuria and glomerulosclerosis (Tullos *et al.* 2015).

Positive cardiorenal effects of ET$_A$ blockade with atrasentan, which were independent of blood pressure, were demonstrated in salt-sensitive Dahl rats kept on high-salt diet for 12 weeks (Samad *et al.* 2015). Although no hypotensive effects of enalapril (10 mg/kg/day) or atrasentan (5 mg/kg/day) were observed at the end of the study, both drugs attenuated cardiac hypertrophy. In addition, atrasentan but not enalapril decreased proteinuria and serum creatinine suggesting the attenuation of CKD progression with ET$_A$ blockade in this model.

A new potential for endothelin antagonists in non-diabetic CKD also derives from their positive effects in acute and chronic kidney damage induced by nephrotoxic agents. While Helmy *et al.* (2014) reported on the beneficial effects of selective ET$_A$ receptor blockade (but not of nonselective blockade) in cisplatin-induced nephrotoxicity, Caires *et al.* (2017) demonstrated positive effects of nonselective blockade with both bosentan and macitentan in reversing cyclosporine-induced changes mainly through their hemodynamic effects. Interestingly, the effects of bosentan were even better than those of macitentan.

The negative side effects of calcineurin inhibitors such as cyclosporine and tacrolimus are associated with the activation of both renin-angiotensin and endothelin systems (Hošková *et al.* 2017). The mechanism underlying nephrotoxic effects of cyclosporine A (CSA) is connected with the induction of endothelin-converting enzyme by CSA resulting in ET-1 generation which leads to further renal hypoxia and ET-1 synthesis (Heyman *et al.* 2018). Blockade of ET$_A$ receptors with atrasentan ameliorated nephrotoxic effects induced by a combination of CSA with indomethacin, including positive effects on renal function and inhibition of oxidative stress (Helmy *et al.* 2015).

Another possible field for the use of ET$_A$ antagonists is nephropathy associated with sickle cell disease (SCD). The first demonstration of positive effects of nonselective ET antagonist bosentan on organ injury and mortality in a mouse model of SCD was reported by Sabaa *et al.* (2008). The mechanisms underlying the involvement of endothelin as a mediator of diverse complications occurring in different organs in sickle cell disease involve the endothelial dysfunction associated with albuminuria (Ataga *et al.* 2016) and the stimulation of ET$_B$ receptors present on neutrophils which leads to their increased adhesion to the endothelium (Koehl *et al.* 2017). Fox and Kasztan in their review (2016) provided a deep insight into this area showing positive results of endothelin receptor antagonists in the treatment of this disease which may lead to the adoption of ET$_A$ blockade as a novel pharmacological strategy. In fact, recent study of Kasztan *et al.* (2017) demonstrated in a murine model of SCD that a ten-week treatment with ambrisentan started from weaning dramatically improved glomerular filtration rate and reduced proteinuria, which was accompanied by an improvement of the podocyte structure, decreased podocyte loss and attenuation of glomerulosclerosis. In contrast, combined ET$_A$/ET$_B$ antagonist (A-182086) provided only a partial protection in comparison with selective ET$_A$ blockade, supporting the rationale of implementing this strategy in the treatment of this type of non-diabetic CKD.

Endothelin-1 plays an important role also in ischemia/reperfusion (IR) injury following coronary ischemic events as it is released at higher levels into the circulation after myocardial infarction (Tamareille *et al.* 2013). It has been shown that ET$_B$ receptors have a critical role in this process. This is caused by the increased sensitivity to ET-1 associated with the augmented ET$_B$ receptor-mediated contractility of vascular smooth muscle cells (Kristiansen *et al.* 2018). However, Boesen *et al.* (2016) did not disclose an effect of ET$_A$ blockade on renal blood flow in mouse after unilateral renal I/R injury in mouse, although it had positive effect on renal mass.

Last but not least, we should mention the positive results of ET$_A$ blockade in pigs with renal artery stenosis – showing that a four-week treatment with ET$_A$ receptor antagonist following percutaneous transluminal renal angioplasty led to a further attenuation of hypertension and renal injury. In contrast, non-selective blockade led to a deterioration of the kidney function (Chade *et al.* 2015).

Positive results from most of the above mentioned experiments together with the DUET study in patients with focal segmental glomerulosclerosis support the use of ET$_A$ antagonists at least in non-diabetic CKD. However, more studies are necessary to confirm this idea.

Importantly, new insights into the complex pathogenetic mechanisms of CKD include the study of Dhande *et al.* (2018) involving the genetic susceptibility to hypertension-induced renal injury in stroke-prone, spontaneously hypertensive rats and indicating that hypertensive kidney damage is associated with a genetic variation of the immunoglobulin heavy chain.

Diuretics in CKD

Chronic kidney disease is often complicated by hypertension which is a major risk factor in CKD progression. Therefore, the control of hypertension is an associated critical goal which should be managed by pharmacological treatment. Diuretics in combination with other classes of antihypertensives, such as RAS blockers, calcium channel blockers, and β blockers, are the inexpensive and efficient second-line treatment possibility which can be administered even in advanced CKD. Although thiazides were not previously recommended in advanced stages of CKD, their involvement is now reconsidered (Sinha and Agarwal 2015) on the basis of clinical studies showing that the addition of thiazides to RAS blockers in advanced CKD is effective in the lowering of blood pressure (Cirilo *et al.* 2014, Fuwa *et al.* 2016) and proteinuria (Morales *et al.* 2015).

The use of ET_A receptor blockade in clinical practice is limited by their side-effect on fluid retention, which is probably of renal origin. Originally, ET-1 was demonstrated to act through ET_B receptors to promote natriuresis and diuresis, especially in response to increased salt load (Ahn *et al.* 2004). In addition to many experimental studies, diuresis and natriuresis following ET-1 infusion has also been recently demonstrated in humans (Hunter *et al.* 2017). Previous studies in rats with knockout of ET_B receptors demonstrated salt-sensitive hypertension in these animals (Gariepy *et al.* 2000). In addition, specific knockout of ET-1 or ET_B receptor genes in the collecting duct led to sodium retention and hypertension (Ahn *et al.* 2004, Ge *et al.* 2006). Later, the contribution of ET_A receptors to the natriuretic response to ET-1 was reported in female ET_B-deficient rats (Nakano and Pollock 2009). Stuart *et al.* (2013) demonstrated the absence of ET_A-induced fluid retention during ambrisentan or atrasentan treatment in mice with a deletion of ET_A receptor in mice (either in the whole nephron or in the collecting duct only) suggesting rather the role of ET_A instead of ET_B receptor in this process.

Recently, experiments in rats demonstrated that the mechanisms of edema formation during selective ET_A blockade result from the activation of ET_B receptors leading to an increase in vascular permeability and fluid retention (Vercauteren *et al.* 2017). Whatever are the mechanisms responsible for the unwanted fluid retention, the use of diuretics to overcome this problem seems to be reasonable. In line with this, Weber *et al.* (2009) demonstrated in patients with resistant hypertension that fluid retention caused by darusentan could be managed with diuretics.

Jin *et al.* (2014) reported on the beneficial renoprotective effects of combined a four-week treatment with thiazide diuretic chlorthalidone with ET_A blockers in a model of metabolic syndrome (Dahl salt-sensitive rats on high-salt and high-fat diet), which was associated with the improvement of podocyte function. The beneficial effect of a combined treatment with angiotensin receptor blocker losartan and diuretic hydrochlorothiazide (Arias *et al.* 2013) (but not with furosemide) was also documented in 5/6 nephrectomized rats (Arias *et al.* 2016). The superiority of hydrochlorothiazide over other diuretics has been documented also in the clinical studies on CKD (Morales *et al.* 2015, Fuwa *et al.* 2016).

Our laboratory demonstrated the positive effects of treatment with diuretic hydrochlorothiazide on top of combined RAS and ET_A blockades in a long-term study performed in adult hypertensive TGR rats subjected to 5/6 nephrectomy (Fig. 1) (Vaněčková *et al.* 2017). We have found that all therapies which included RAS blockade substantially improved survival of rats with experimentally induced CKD. Moreover, the addition of an ET_A receptor blockade had further beneficial effect. The renal damage evaluated by proteinuria gradually increased in rats treated with RAS blockade alone (green line), while it was substantially lowered by additional ET_A blockade (blue line).

Most importantly, in rats treated with a diuretic added to a combination therapy (hydrochlorothiazide on top of RAS and ET_A blockade – red line) proteinuria was progressively reduced throughout the duration of the experiment. Finally, since blood pressure was maximally reduced by RAS therapy in all groups, it is evident that this effect is blood pressure-independent and goes beyond that of selective ET_A blockade. However, it must be proven in further studies whether this "triple" therapy will also be successful in animals with already established CKD.
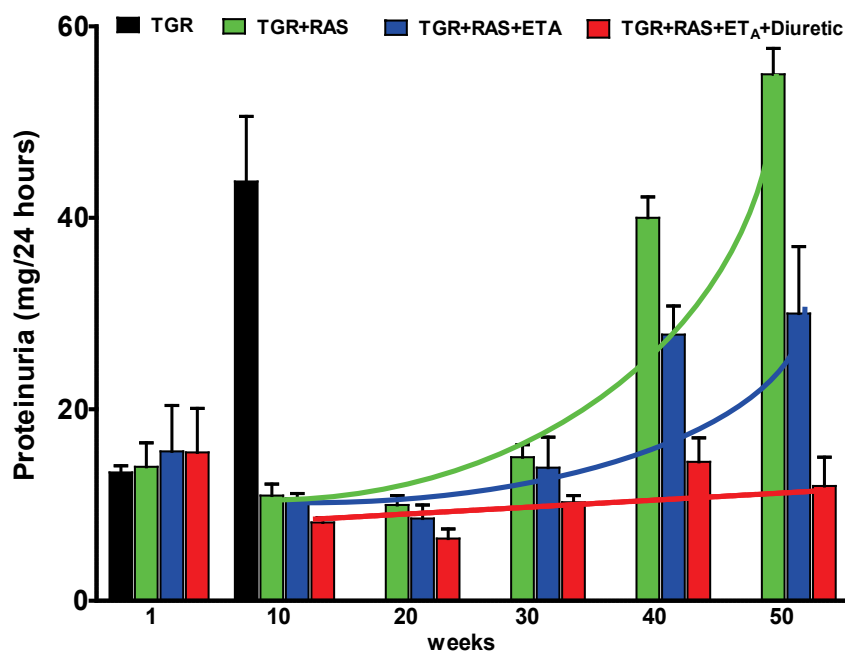
Fig. 1. Proteinuria in 5/6 nephrectomized (black bars) Ren-2 transgenic rats during a 50-week treatment with RAS blockers alone (trandolapril and losartan) (green bars), RAS blockade + ET_A antagonist (atrasentan) (blue bars), and RAS blockade + ET_A blockade + diuretic (hydrochlorothiazide) (red bars). Untreated animals died within 18 weeks following 5/6 nephrectomy.

Since the sites and mechanisms of natriuretic action of thiazide diuretics (Na^+Cl^- cotransporter in the distal tubule) and ET-1 (mainly epithelial sodium channel in the cortical collecting duct) (Lynch *et al.* 2013, Kohan 2013) are different, the exact mechanism of positive results of the treatment with thiazide diuretics in a model of CKD in TGR rats needs to be examined in the future.

Conclusions

Although the major clinical trials evaluating endothelin receptors in diabetic patients were prematurely terminated, there are experimental data in non-diabetic chronic kidney disease supporting the potential of endothelin receptor blockers at least under these conditions.

However, as with all drugs, caution must be given to their side-effects – in case of ET_A receptors –
fluid retention. However, it appears that this problem could be overcome; based on our experimental study diuretic treatment added to RAS and ET_A receptor blockade could further attenuate renal damage. Considering the positive results of the DUET trial in patients with focal segmental glomerulosclerosis as well as experimental studies in non-diabetic CKD, there is still hope for future advancement in this field.

Conflict of Interest
There is no conflict of interest.

Acknowledgements

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