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Dual inhibition of renin-angiotensin-aldosterone system and endothelin-1 in treatment of chronic kidney disease

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Komers R, Plotkin H. Dual inhibition of renin-angiotensin-aldosterone system and endothelin-1 in treatment of chronic kidney disease. *Am J Physiol Regul Integr Comp Physiol* 310: R877–R884, 2016. First published March 23, 2016; doi:10.1152/ajpregu.00425.2015.—Inhibition of the renin-angiotensin-aldosterone system (RAAS) plays a pivotal role in treatment of chronic kidney diseases (CKD). However, reversal of the course of CKD or at least long-term stabilization of renal function are often difficult to achieve, and many patients still progress to end-stage renal disease. New treatments are needed to enhance protective actions of RAAS inhibitors (RAASis), such as angiotensin-converting enzyme (ACE) inhibitors (ACEIs) or angiotensin receptor blockers (ARBs), and improve prognosis in CKD patients. Inhibition of endothelin (ET) system in combination with established RAASis may represent such an approach. There are complex interactions between both systems and similarities in their renal physiological and pathophysiological actions that provide theoretical rationale for combined inhibition. This view is supported by some experimental studies in models of both diabetic and nondiabetic CKD showing that a combination of RAASis with ET receptor antagonists (ERAs) ameliorate proteinuria, renal structural changes, and molecular markers of glomerulosclerosis, renal fibrosis, or inflammation more effectively than RAASis or ERAs alone. Practically all clinical studies exploring the effects of RAASis and ERAs combination in nephroprotection have thus far applied add-on designs, in which an ERA is added to baseline treatment with ACEIs or ARBs. These studies, conducted mostly in patients with diabetic nephropathy, have shown that ERAs effectively reduce residual proteinuria in patients with baseline RAASis treatment. Long-term studies are currently being conducted to determine whether promising antiproteinuric effects of the dual blockade will be translated in long-term nephroprotection with acceptable safety profile.

angiotensin II; chronic kidney disease; diabetic nephropathy; endothelin; FSGS

INHIBITION of the renin-angiotensin-aldosterone system (RAAS) plays a pivotal role in treatment of chronic kidney diseases (CKD). Inhibitors of the RAAS (RAASis) can slow the progressive decrease in glomerular filtration rate (GFR), reduce proteinuria, and cardiovascular mortality and morbidity in both diabetic and nondiabetic proteinuric kidney diseases. However, despite documented beneficial effects of RAASis, reversal of the course of progressive forms of CKD or at least long-term stabilization of renal function are often difficult to achieve, and many patients still progress to end-stage renal disease (ESRD). New approaches that would broaden the spectrum of available treatments or enhance protective actions of RAASis are needed to improve prognosis in these patients. As indicated by evidence collected over the past two decades, parallel inhibition of the RAAS and endothelin (ET) system may represent such an approach. In this review we will discuss whether there is

evidence supporting this view. Basic physiology and pathophysiology of both systems in the kidney have been extensively studied and have been the subject of numerous experimental and clinical reports including excellent reviews. In this paper we will focus only on data relevant for the topic of dual inhibition of both systems in the treatment of kidney disease.

RAAS-Endothelin-1 Interface in Kidney

RAAS in renal physiology and pathophysiology. Main effectors of RAAS, such as angiotensin II (ANG II) or aldosterone, have well-established actions in the kidney and roles in renal pathophysiology (63, 81). In brief, ANG II, acting mostly via AT_1 receptors, affects practically all renal compartments and cell types. These effects include hemodynamic actions leading to vasoconstriction and elevations of intraglomerular pressure; promoting cell growth and extracellular matrix (ECM) production resulting in glomerulosclerosis and tubulointerstitial fibrosis (TIF); prooxidant and inflammatory actions as well as effects with implications in podocyte pathophysiology and

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pathogenesis of proteinuria. Similarly, aldosterone has prosclerotic, fibrogenic, and "proteinuric" effects, in addition to its principal roles in the control of sodium/potassium homeostasis and blood pressure (BP) (74, 76). Inhibition of RAAS leads to at least partial suppression of those actions during the development and progression of kidney disease.

Endothelin-1 in renal physiology and pathophysiology. Some actions of RAAS effectors, in particular those of ANG II, resemble renal actions of endothelin-1 (ET-1), another peptide implicated in renal pathophysiology, and the most important of ET peptides with respect to renal physiology. ET-1 has been also well established as a player in renal pathophysiology. It is stimulated by numerous factors known to trigger or to contribute to the development of kidney diseases (summarized in Ref. 40). In general, ET-1 acts as a vasoactive peptide, which also stimulates renal cell growth, proliferation, production of ECM, and inflammation (40) and has major impact on tubular function (42). In the following sections we will briefly review actions of ET-1 with respect to individual renal cell types and compartments and point out parallels as well as important differences compared with RAAS effectors.

Effects of ET-1 in the renal vascular tree. Similar to ANG II, ET-1 is involved in the control of renal hemodynamics. Actions of ET peptides in the kidney are mediated by ET_A and ET_B receptors (reviewed in Ref. 42). Both ET_A and ET_B receptors on vascular smooth muscle cells mediate ET-1-induced vasoconstricton, whereas ET_B, localized on endothelial cells, mediates endothelium-dependent vasodilation. The effects of ET-1 on the renal vascular tree are complex and segment specific. Studies in different experimental settings indicate that ET-1 is preferentially a preglomerular vasoconstrictor (summarized in Ref. 42), although this is not a uniform finding, with some species specificity (46). In addition to its effects on vascular tone, the peptide causes endothelial dysfunction, vascular hypertrophy, and remodeling as observed in both hypertensive (3) and normotensive (15) models of kidney disease.

Glomerular effects of ET-1. ET-1 and ANG II have similar impact in glomeruli, as it happens with vascular actions. ET-1 has been implicated in mesangial cell contraction, proliferation (72), and ECM production (73). ET-1 actions may result in glomerulosclerosis (discussed in *Endothelin-1 promotes the development of glomerulosclerosis, TIF, and renal inflammation*). There is increasing evidence that ET-1 plays specific roles in podocyte alterations, apoptosis, and loss, nephrin shedding, being implicated in loss of synaptopodin and cytoskeletal rearrangement resulting in foot process effacement, a hallmark of podocytopathies (7, 12, 47, 66) including focal segmental glomerulosclerosis (FSGS) (12, 17). Podocytes are not only targets of ET-1 actions, but also a source of the peptide with deleterious effects on adjacent glomerular endothelial cells, causing mitochondrial dysfunction and production of reactive oxygen species (17).

In the context of podocyte changes and loss of podocytic proteins, Saleh et al. (65, 67) described blood pressure-independent, ET-1-induced enhanced glomerular permeability to albumin both in vitro and in vivo. Altogether, these actions contribute to the proteinuric effects of ET-1. Most of these glomerular actions are mediated via ET_A receptors, albeit a recent study suggests the possible pathophysiological role of ET_B expressed on podocytes (47). Treatment with ET receptor antagonists (ERAs) (12, 17, 56), or more recently by genetic deletion of ET receptors (ETRKO) in models of podocyte injury, ameliorated cytoskeletal changes and restored podocyte structural integrity in parallel with reduction in proteinuria (47).

Endothelin-1 promotes the development of glomerulosclerosis, TIF, and renal inflammation. While in the glomeruli ET-1 actions may result in glomerulosclerosis, in the tubulointerstitial compartment, ET-1 triggers processes leading to TIF, both processes being linked to renal inflammation (78). They are, therefore, discussed together in this section. The role of ET-1 in the above-mentioned processes has been recognized for two decades. As shown by Hocher et al. (32) transgenic mice overproducing ET-1 develop glomerulosclerosis and interstitial fibrosis and inflammation without concurrent hypertension, suggesting that elevation in ET-1 contributes to renal fibrosis.

Most of the evidence about these effects of ET-1 has been provided by numerous studies demonstrating beneficial effects of ET-1 receptor inhibition or ETRKO in models of kidney diseases that lead to glomerulosclerosis and/or TIF. Acting mostly via ET_A receptors, ET-1 has been implicated in increased expression and activity of well-established proinflammatory and profibrotic signaling molecules and mediators, including nuclear factor-κB (NFκB) (27, 47, 78), MCP-1, interleukin-6, adhesion molecules (64, 65, 69, 73, 86), transforming growth factor-β (TGF-β) (25, 66, 69, 78), connective tissue growth factor (CTGF) (78), as well as ECM proteins (10, 25, 73, 78). These molecules have also been traditionally implicated in renal pathophysiological actions of ANG II (34, 35, 85) or aldosterone (29, 30, 63). The prosclerotic/fibrogenic and inflammatory actions of ET-1 may occur as part of ANG II signaling (63), or as a direct consequence of stimulation of ET_A receptors (65, 73).

Effects of ET-1 on tubular function. Importantly, there are also opposing actions of RAAS effectors and ET-1 in the kidney, in particular in their net effect on Na homeostasis and extracellular fluid control.

Endothelin-1 is synthesized by and binds to all tubular segments to regulate ion transport. However, unlike antinatriuretic actions of ANG II or aldosterone, ET-1 acts as a natriureric peptide. The major site of natriuretic and diuretic actions of ET-1 is in the collecting duct (CD), which is also the predominant site of ET-1 tubular production (39). In the CD, ET-1 inhibits epithelial Na channel (ENaC), one of aldosterone-responsive transporters, acting via multiple signaling pathways (28, 38, 59, 70). Adding complexity, aldosterone has been shown to stimulate ET-1 production in the CD (84), raising a possibility that ET-1-induced inhibition of ENaC represents a feedback mechanism to mitigate aldosterone actions. ET-1 also inhibits Na^+-K^+-Cl^- cotransporter (NKCC2) in thick ascending limb (31) and activates $Na^+/H+$ exchanger (NHE3) in the proximal tubule (45, 48), and the latter effect is also involved in the control of acid-base balance (48). The peptide also decreases arginine vasopressin (AVP)-stimulated water transport further enhancing diuretic actions (26, 55).

Most of the evidence suggests that ET_B is the main receptor responsible for natriuretic actions of ET-1. However, observations in mice with double ET_A/ET_B knockout in the CD, which display more severe hypertension than ET_B knockout mice, indicate a contribution of ET_A to ET-1-induced natriuresis (reviewed in Ref. 42). This is further supported by the fact that

edema, fluid retention, or even heart failure complicate clinical use of the selective ERAs (50).

Interactions and cross-talk between RAAS and ET-1. In addition to similarities in some actions of ET-1 and effectors of RAAS in the pathophysiology of kidney diseases, there are complex interactions and cross-talk between both systems. ANG II stimulates ET-1 release and expression in a variety of cell types including renal cells (23, 24, 43). ET-1 mediates some of the vascular actions of ANG II. ANG II-dependent increases in ET-1 contribute to the vasoconstrictor effects of ANG II in vitro in isolated vascular preparations (16, 79) and in vivo [e.g., in the skin microcirculation in healthy humans (83)]. ET receptor blockade can inhibit the acute vasoconstrictor responses to ANG in vivo, including within the renal circulation (6, 61). In turn, ET-1 stimulates ANG II formation possibly via enhanced action of ACE in vitro in pulmonary endothelial cells (36, 37). Whether ET-1 stimulates ANG II also in nonvascular and renal cells remains to be established.

ET-1 also stimulates aldosterone secretion, as well as zona glomerulosa cell growth and proliferation (52, 53). Nonselective ET receptor blockade decreases plasma aldosterone concentration, zona glomerulosa proliferation, and aldosterone release by zona glomerulosa preparations in rats transgenic for the renin gene (2). Furthermore, in humans with high to normal renin hypertension, ET_A or combined $ET_{A/B}$ blockade reduces plasma aldosterone (62).

In contrast to the effects of ET-1 on ANG II and aldosterone, ET-1 appears to inhibit release of renin. ET-1 inhibits renin release from isolated juxtaglomerular apparatus (51, 60). In vivo, low doses of ET-1 that do not produce any significant changes in total peripheral resistance also reduce renin release as observed in anesthetized dogs (49, 57). Higher doses of ET-1 that increase BP increase plasma renin activity (57). However, it is questionable whether this observation is physiologically relevant considering high BP-induced adaptations in renal hemodynamics that may stimulate renin release. The relationship between ET-1 inhibiting the rate-limiting step in the RAAS activation cascade and parallel enhancement of the actions of its downstream effectors remains unknown. Some authors speculate that ET-dependent inhibition of renin release promotes sodium excretion as part of natriuretic physiological functions of ET-1 (42).

Interactions of ET-1 with another effector of RAAS, angiotensin-(1–7), have been also described in experimental settings (9), but their clinical relevance remains to be established and will not be a subject of this review.

Does Experimental Evidence Support Superior Effects of Dual RAAS-ET-1 Inhibition in Treatment of Kidney Disease Compared With Monotherapies With RAAS or ET-1 Antagonists?

Altogether, there is persuasive evidence for similarities of actions of RAAS effectors and ET-1 in processes that trigger or perpetuate renal injury. This provides rationale for testing possible additive effects of dual inhibition of both systems in treatment of kidney disease.

The concept of dual inhibition of ANG II and ET-1 in nephroprotection is not new and has been developing since the 1990s. Benigni et al. (8) first studied dual blockade in passive Heyman nephritis, a model of idiopathic membranous ne-

phropathy. The combination treatment with angiotensin-converting enzyme inhibitor (ACEI) trandolapril and ET_A antagonist LU-135252 had no additive effects on BP compared with trandolapril alone, but the combination was the only treatment that induced significant reduction of proteinuria compared with untreated passive Heyman nephritis animals, whereas the antiproteinuric effect ET_A inhibitor or ACEI in monotherapies was not significant. The combination also prevented the rise in serum creatinine (S-Cr) consistent with superior preservation of kidney function at the end of the follow-up (8 mo). Unlike the monotherapies, the combination attenuated the development of glomerulosclerosis. Amann et al. (1) combined ERA LU-135252 with angiotensin receptor blocker (ARB) fonsartan or ACEI ramipril and compared their effects to each monotherapy in 5/6th nephrectomized rats, a model of secondary FSGS. The combination of ERA with ARB was most effective in preventing the development of TIF, albeit this study did not find significant differences between the animals treated with the combination and ACEI or ARB monotherapies on proteinuria, BP, and glomerulosclerosis. Similarly, atrasentan combined with ACEI trandolapril or losartan was more effective than monotherapies in another hypertensive model, 5/6th nephrectomized rats with overexpression of renin gene, when administered immediately after the kidney mass reduction (14).

In experimental diabetic nephropathy, Gagliardini et al. (25) evaluated the effect of simultaneous inhibition of ET-1 with avosentan and ACEI lisinopril in uninephrectomized STZ-diabetic rats. The treatments were applied in monotherapy or in combination between 4 to 8 mo after streptozotocin (STZ) injection. Combined therapy corrected proteinuria, ameliorated several markers of tubulointerstitial damage, and induced regression of glomerular lesions, while only a partial renoprotection was achieved by each drug alone. The combination restored the number of podocytes while monotherapies only limited podocyte depletion. Defective nephrin expression in diabetes was prevented by both drugs. Altered glomerular size selectivity to large macromolecules of diabetic rats was remarkably improved by lisinopril and the combined treatment. Avosentan ameliorated changes in peritubular capillary architecture and reduced tubular damage score, interstitial inflammation as well as expression of TGF-β and collagen III.

It should be noted that not all studies support physiologically relevant additive effects of ERAs and RAASis. In a study by Cao et al. (13), ACEI perindopril or ARB irbesartan, or their combination, markedly attenuated development of hypertension, proteinuria, loss of GFR, renal structural changes, and molecular markers of nephropathy such as TGF-β or collagen IV mRNA expression in 5/6th nephrectomized rats. These beneficial effects were not observed in rats treated with s BMS193884 or with a nonselective ET-1 receptor blocker bosentan, unlike more recent studies by Amman et al. (1). Moreover, ET-1 antagonists did not enhance any of the abovementioned protective actions of RAASis. In Zucker diabetic fatty rats, a model of Type 2 diabetes (87), ACEI ramipril was more effective compared with ERA sitaxsentan in reduction of BP, albuminuria, glomerulosclerosis score, renal collagen III expression, and markers of intrarenal inflammation. The study failed to demonstrate any additive protective effects with either agent in this model, albeit the authors conclude that adding ERA to ACEI slightly enhances antiinflammatory actions of ACEI. Finally, as very recently observed, the superior protec-

tive effects of dual blockade in 5/6th nephrectomized Ren-2 rats discussed above (14) were not apparent when the treatment was administered several weeks after kidney mass reduction (V. Certikova-Chabova, personal communication).

Effects of Dual Inhibition in Humans With CKD

Practically all clinical studies published thus far in this field have add-on designs, in which ERAs is added to baseline treatment with ACEIs or ARBs. Most of the studies have focused on reduction of residual proteinuria in patients with baseline RAASis treatment.

Additive effects on proteinuria were initially noted in acute studies with ERAs in patients with nondiabetic CKD caused by various forms of glomerulonephritis and secondary FSGS. Infusion of BQ123 reduced proteinuria more than nifedipine (21), despite similar effects on BP. This effect was more prominent in patients pretreated with ACEIs or ARBs compared with ACEIs alone (22). The authors later reported a more prolonged observation (6 wk) in a similar cohort of patients with primary and secondary nondiabetic glomerulopathies and baseline treatment with ACEIs or ARBs (20). The patients received sitaxsentan, nifedipine, or placebo. Unlike placebo, add-on sitaxsentan further decreased proteinuria, BP, and induced changes in renal hemodynamics characterized by lower GFR and no change in renal plasma flow (RPF) resulting in a substantial decrease in filtration fraction (FF). Except comparable reduction in BP, the patients treated with nifedipine did not demonstrate changes in proteinuria or renal hemodynamics. These data suggested hemodynamically mediated antiproteinuric effect of sitaxsentan.

In a study by Weber et al. (80), investigators focused on the effects of ERA darusentan in patients with resistant hypertension, but the treatment-induced changes in albuminuria were also determined. The study is relevant since 96–99% of patients were in parallel treated with ARBs or ACEIs. Type 2 diabetes was present in about 40% of patients. Darusentan was administered at three dose levels (100, 200, and 300 mg/day) for 14 wk. The two higher doses lead to a significant reduction in albuminuria compared with placebo-treated patients who received only RAASis.

The antiproteinuric effects of dual blockade have been thus far best studied in patients with diabetic nephropathy. In initial studies, ERA avosentan was used to assess the effects of ERA on residual proteinuria. In a short-term (12 wk) phase 2a trial, avosentan (5–50 mg/day) reduced albuminuria by up to 40% compared with placebo in Type 2 diabetic patients with nephropathy, who were in most cases (~95%) treated with maximal doses of RAASis (82). The effect was independent of the avosentan dose. In contrast to substantial antiproteinuric effect, there were no changes in BP in avosentan-treated patients. Dose-dependent fluid retention was noted as major adverse event associated with avosentan. The ASCEND (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) trial (50) was designed to study avosentan in a large cohort of diabetic patients with maximal doses of RAASis with a focus on hard endpoints such as composite of doubling of S-Cr, initiation of

renal replacement therapy and death. The trial was stopped prematurely after 4–5 mo because of excess cardiovascular adverse events associated with avosentan treatment (25 and 50 mg/day), mainly congestive heart failure and fluid overload. Yet, the trial confirmed strong beneficial effect of avosentan on residual proteinuria, reaching 40% further reduction compared with placebo.

More recent studies in this area have been conducted with atrasentan, a highly selective ERA. In a phase 2a study, the effects of atrasentan (0.25, 0.75, and 1.75 mg/day) on albuminuria compared with placebo were assessed for 8 wk in 89 Type 2 diabetic subjects with diabetic nephropathy and moderate albuminuria receiving stable doses of RAASis (41). Reduction in albuminuria was observed in patients treated with higher doses of atrasentan (0.75 and 1.75 mg/day) and reached ~40%, whereas the 0.25 mg/day dose was without any effect. As in previous trials, dose-related occurrence of edema was reported, being 9, 14, 18, and 46% in placebo, 0.25, 0.5, and 1.75 mg/day atrasentan groups, respectively.

The Reducing Residual Albuminuria in Subjects With Diabetes and Nephropathy With AtRasentan (RADAR) trial (18) that followed has been thus far the largest and best documented study assessing dual blockade in proteinuric CKD. The study tested atrasentan at 0.75 and 1.25 mg/day and did not include the highest dose from the previous study (41), in Type 2 diabetic patients with albuminuria [albumin-to-creatinine ratio (ACR) 500-3,500 mg/g] and eGFR within the 30–75 ml/min range treated with maximally tolerated baseline ACEIs or ARBs. In contrast to placebo, both doses of atrasentan were effective in reducing residual albuminuria by about 40%. There was mild increase in body weight, but not in prevalence of edema, and the treatment was in general well tolerated.

In both studies, atrasentan also reduced BP. This modest add-on effect of atrasentan on BP can hardly explain impressive effects on residual proteinuria. On the other hand, rapid return of albuminuria after drug withdrawal (as observed in the atrasentan studies) suggests contribution of the hemodynamic mechanism to the antiproteinuric effect, in accord with a previous report by Dhaun et al. (20), which showed ERA-induced changes in whole kidney renal function parameters suggestive of changes in glomerular hemodynamics. Detailed measurements of renal hemodynamics were not performed in the atrasentan studies and modest changes in FF could have remained undetected. The decrease in albuminuria after initiation of atrasentan treatment was rapid as was the increase to baseline values in this parameter after discontinuation of the treatment. This further supports a renal hemodynamic mechanism of atrasentan antiproteinuric actions in those trials.

For completeness, the list of this type of studies should include a recently published study exploring the effects of dagutril, an inhibitor of neutral endopeptidase and ET-converting enzyme, in albuminuric Type 2 diabetic patients treated with losartan (58). Despite an effect on BP, this treatment did not influence albuminuria over a period of 8 wk. The treatment decreased ET generation, but due to a different mechanism of action it could be hardly compared with more specific ETA inhibition.

Perspectives and Significance

A spectrum of issues remain be addressed in future or ongoing studies: Most importantly, it remains unknown
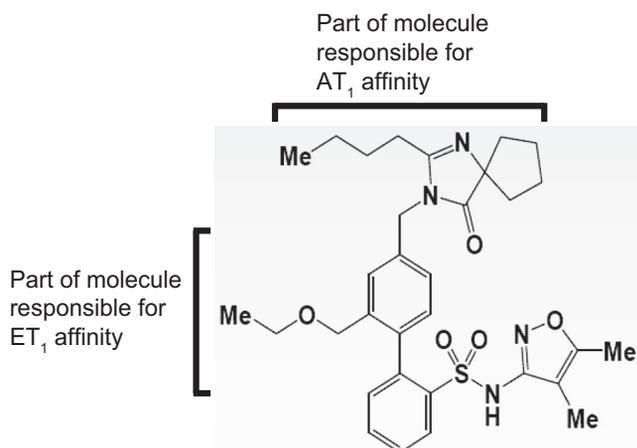
Fig. 1. Chemical structure of sparsentan. The figure shows chemical structure of sparsentan with parts of the molecule important for affinities to AT1 and ETA receptors. Adapted with permission (44).

whether promising signals from the above-discussed studies can be translated into long-term nephroprotection. Moreover, it is not known whether dual blockade is more beneficial than monotherapies on cardiovascular endpoints in patients with CKD.

It has not been well established how much of the antiproteinuric effect is attributable to BP lowering or changes in renal hemodynamics achieved by the combination. Available clinical studies do not offer unequivocal evidence, albeit it could be argued that modest add-on effects of ERAs in most studies on BP can hardly explain impressive effects on residual proteinuria. On the other hand, rapid return of albuminuria after the drug withdrawal as observed in atrasentan studies (18, 41) suggests contribution of a hemodynamic mechanism to the antiproteinuric effect. In addition, direct effects of ET-1 on glomerular permeability discussed in previous sections (65, 67), which are independent of hemodynamic influences, could contribute to both rapid onset of antiproteinuric effect in response to ERAs as well as to rapid return of albuminuria after their withdrawal. This rapid return of albuminuria may also indicate the fact that despite marked reduction in proteinuria, the treatment does not appear to have long-term benefit in preserving renal architecture, emphasizing the need for long-term trials.

Use of RAASis is limited in patients with lower GFR because of increased risk of acute renal failure and hyperkalemia, which may be precipitated by critical decrease in glomerular filtration pressure. Therefore, it will clinically be important to determine whether parallel ET-1 inhibition could attenuate decreases in glomerular pressure induced by RAASis and extend the period during which RAASis could be safely used in patients with low GFR.

Considering the effects of ERAs on aldosterone secretion, it will be also interesting to determine whether the dual blockade could ameliorate aldosterone escape, a phenomenon observed in some patients treated with RAASis and implicated in partial loss of efficiency of ACEIs or ARBs (11).

SONAR trial. Some answers, in particular about the potential of dual blockade in long-term nephroprotection, may be provided by the ongoing study of diabetic nephropathy with atrasentan (SONAR) trial (16b). This is a phase 3 prospective,

randomized, multicenter, double-blind, parallel, placebo-controlled study of the effects of atrasentan on renal outcomes in Type 2 diabetic subjects with nephropathy. The investigators will randomize more than 4,000 patients with eGFR 25 to 75 $ml \cdot min^{-1} \cdot 1.73 \ m^{-2}$ and a ACR $\geq$ 300 and < 5,000 mg/g, treated with maximal tolerated dose of ARBs or ACEIs, to receive atrasentan 0.75 mg/day or placebo. The double-blind treatment period is estimated to continue for ~48 mo. The primary endpoint is time to the first occurrence of a component of the composite renal endpoint: doubling of S-Cr or the onset of ESRD (needing chronic dialysis, renal transplantation or renal death). In addition to the primary endpoint, secondary efficacy endpoints will focus on reduction in proteinuria, effects on the rate of decline in eGFR, and cardiovascular endpoints such as nonfatal myocardial infarction or stroke.

Dual AT_1 and ET_A receptor antagonists. Studies discussed thus far had uniformly applied add-on design to test dual RAAS and ET-1 inhibition, i.e., a combination of individual receptor blockers. In addition to this approach, investigators have been developing dual AT_1 and ET_A receptor antagonists, i.e., single molecules that can inhibit both receptors. The synthesis of dual ANG II and ET receptor antagonists was based on recognition of the structural similarities between irbesartan (an ARB) and some ET_A receptor antagonists (such as BMS 193884) (54)–a resemblance of the core biphenyl framework of these ET_A antagonists to the biphenyltetrazole core characteristic for a spectrum of AT_1 receptor antagonists, including irbesartan. It was hypothesized that merging the structural elements of these two antagonists would yield a compound with dual activity for both receptors. This strategy led to the design, synthesis, and discovery of potent and orally active antagonists of both AT_1 and ET_A receptors (dual action receptor antagonists, DARA). Detailed synthetic process and chemistry of these compounds has been described in the
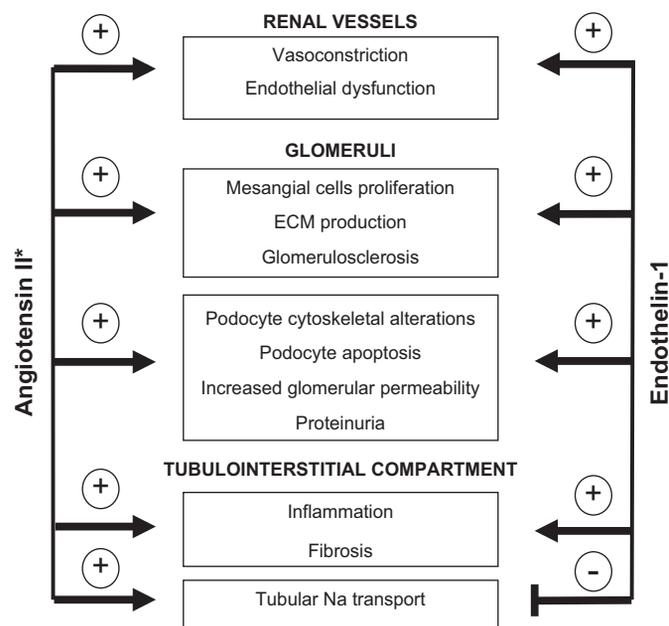

Fig. 2. Schematic presentation of major similarities and differences in physiological and pathophysiological actions of angiotensin II and endothelin 1 in the kidney. Both peptides have similar impact on processes implicated in renal pathophysiology. In contrast they differ in their role in the control of tubular transport. More detail in text. *Applies also to aldosterone.

literature (44, 54). These DARA inhibited ^{125}I-labeled Sar-Ile-ANG II binding to rat or human AT_1 receptors and ^{125}I-labeled ET-1 binding to rat or human ET_A receptors in a concentration-dependent manner. The compounds were inactive against human AT_2 and ET_B receptors as well as many other G protein-coupled receptors, enzymes, and ion channels.

Kowala et al. (44) reported inhibition constants (K_i), calculated from the IC_{50} values, for inhibiting ^{125}I-labeled Sar-Ile-ANG II binding to AT_1/AT_2 receptors and ^{125}I-labeled ET-1 binding to ET_A/ET_B receptors. K_i of one of these compounds, later called sparsentan (Fig. 1), for human AT_1 receptor was 0.8 ± 0.1 and for human ET_A receptor 9.3 ± 1.1, whereas the affinity toward AT_2 and ET_B receptors was negligible ($>1,000$). For comparison, the values for K_i of irbesartan for the K_i of irbesartan for AT_1 was 1.1 ± 0.3. Further evidence for the dual antagonistic action of DARA compounds was demonstrated by their ability to reduce acute increases of blood pressure in rats caused by bolus intravenous infusions of either ANG II or BigET-1 (54). Moreover, the DARA were more effective in reducing blood pressure compared with equimolar dose of irbesartan in models of hypertension such as spontaneously hypertensive rats and Dahl salt-sensitive rats (44).

Sparsentan was further developed by Pharmacopeia and Ligand as a promising antihypertensive agent. There were seven phase 1 clinical studies performed in healthy volunteers and two phase 2 studies in patients with essential hypertension. The results of these studies have not been published. In brief, although the focus of those studies were not on proteinuria, they were helpful to understand the generally positive safety profile of the drug and its effects on hypertension, which were stronger than those of irbersartan.

DUET trial. Efficacy of sparsentan is currently explored in the DUET trial, a randomized, double-blind, active-control, dose-escalation study evaluating the antiproteinuric efficacy and long-term safety of sparsentan in patients with primary FSGS (16a). The study has been designed to test the hypothesis that in these patients, sparsentan lowers proteinuria to a greater degree than an ARB irbesartan alone while maintaining a favorable drug safety profile. Patients, age 8–75 yr old, with baseline eGFR > 30 ml/min and Up/c > 1 g/g with primary or genetic forms of FSGS are eligible to participate in the study. The patients are randomized after 2 wk washout of RAASis to receive either sparsentan (200, 400, and 800 mg/day) or irbesartan (300 mg/day) as an active control. This double-blind period will last for 8 wk followed by an open-label phase treatment with sparsentan (144 wk) to assess long-term safety. The primary endpoint is change in protein-to-creatinine ratio (Up/c) from baseline to 8 wk postrandomization. Secondary objectives will focus on metabolic effects of sparsentan, RAAS activity, long-term safety, and quality of life.

Other potential targets for dual RAAS and ET-1 inhibition. Experimental evidence together with emerging case reports suggest that there is a spectrum of disorders that might be particularly responsive to dual inhibition but have not yet been systematically tested in clinical trials. These conditions include renal involvement in sickle cell anemia (3, 75) and scleroderma crisis (19, 33, 71). IgA nephropathy appears also as a promising new target. A special note should be reserved to the potential of dual blockade in preeclampsia. Both ANG II and ET-1 have been implicated in pathogenesis of this disorder (68,

77). However, because of teratogenic potential, ARBs, ACEIs, and in particular, ET-1 receptor antagonists, are contraindicated during pregnancy. The solution might be provided by the development of inhibitors that do not cross the placental barrier.

In conclusion, dual inhibition of the RAAS and endothelin ET_A receptor emerges as a promising approach to enhance the spectrum of beneficial effects of RAASis in patients with CKD (Fig. 2). In addition to the theoretical rationale for combination suggested by basic research, persuasive clinical evidence currently exists for additive antiproteinuric effect of the dual blockade in patients with diabetic nephropathy. Whether these promising effects could be translated into long-term nephroprotection, i.e., preservation of kidney function and renal architecture, suggested by some experimental studies, needs to be further tested in appropriately designed clinical trials. These trials should also focus on long-term safety, in particular with respect to fluid retention, and on the impact of dual blockade on cardiovascular disease, the major cause of mortality in patients with CKD. The SONAR trial is expected to provide some important data in this context. In the clinical arena, there is still little evidence about the therapeutic potential of dual blockade in nondiabetic proteinuric kidney diseases. This gap is being currently filled by ongoing DUET trial in primary FSGS, but application of the dual blockade in a spectrum of other indications that have been shown to be responsive separately to RAAS or ET-1 inhibition still warrants clinical testing.

DISCLOSURES

AUTHOR CONTRIBUTIONS

Author contributions: R.K. prepared figures; R.K. and H.P. drafted manuscript; R.K. and H.P. edited and revised manuscript; R.K. and H.P. approved final version of manuscript.

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