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Angiotensin II Blockade and Renal Protection

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Abstract

Current national guidelines have recommended the use of renin-angiotensin system inhibitors, including angiotensin II type 1 receptor blockers (ARBs), in preference to other antihypertensive agents for treating hypertensive patients with chronic kidney disease. However, the mechanisms underlying the renoprotective effects of ARBs are multiple and complex. Blood pressure reduction by systemic vasodilation with an ARB contributes to its beneficial effects in treating kidney disease. Furthermore, ARB-induced renal vasodilation results in an increase in renal blood flow, leading to improvement of renal ischemia and hypoxia. ARBs are also effective in reducing urinary albumin excretion through a reduction in intraglomerular pressure and the protection of glomerular endothelium and/or podocyte injuries. In addition to blocking angiotensin II-induced renal cell and tissue injuries, ARBs can decrease intrarenal angiotensin II levels by reducing proximal tubular angiotensinogen and production of collecting duct renin, as well as angiotensin II accumulation in the kidney. In this review, we will briefly summarize our current understanding of the pharmacological effects of an ARB in the kidney. We will also discuss the possible mechanisms responsible for the renoprotective effects of ARBs on type 2 diabetic nephropathy.

Keywords

Angiotensin II; kidney; angiotensin II type 1 receptor blocker; renal protection; hypertension; chronic kidney disease

I. Introduction

Angiotensin II (Ang II) directly constricts vascular smooth muscle cells, stimulates aldosterone production, activates the sympathetic nervous system and increases sodium reabsorption, all of which are mediated through Ang II type (AT) 1 receptor activation and contributes to the development of hypertension [1, 2]. In addition to its hypertensinogenic effect, locally produced Ang II in the kidney activates multiple intracellular signaling pathways and induces inflammation, renal cell growth, mitogenesis, apoptosis, migration and differentiation [1, 2]. These effects of Ang II are also mediated through AT1 receptor activation and play an important role in the pathogenesis of renal tissue injury.

Blood pressure reduction induced by systemic vasodilation with an AT1 receptor blocker (ARB) essentially contributes to its beneficial effects on renal injury during the development of hypertension [3]. Furthermore, ARB-induced renal vasodilation results in an increase in renal blood flow (RBF), leading to improvement of renal ischemia and hypoxia [4, 5]. ARB

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is also effective in reducing urinary albumin excretion rate, independent of blood pressure reduction [6, 7]. Because the raised urinary albumin excretion rate is a strong independent risk factor for end-stage renal disease (ESRD) and cardiovascular events in patients with chronic kidney disease (CKD) [8], treatment with an ARB is now standard practice in hypertensive patients with CKD [9-11]. It is also well established that ARBs exert renoprotective effects beyond their blood pressure lowering effects [12, 13]. Indeed, there is also substantial preclinical evidence that ARBs can inhibit renal cell and tissue injuries induced by locally produced Ang II [2]. Finally, ARBs can decrease intrarenal Ang II levels by reducing proximal tubular angiotensinogen (AGT) and production of collecting duct renin, along with Ang II accumulation in the kidney [1]. Thus, mechanisms underlying the renoprotective effects of ARBs are multiple and complicated.

In this review, we will briefly summarize our current understanding of the pharmacological effects of an ARB in the kidney. We will also discuss the mechanisms responsible for the renoprotective effects of an ARB during the early and late phases of diabetic nephropathy.

2. Pharmacological Effects of ARBs in the Kidney

2-1. Effect of an ARB on Renal Hemodynamics

In this section, effects of an ARB on RBF and glomerular filtration rate (GFR) are briefly discussed. Physiological roles of Ang II in the regulation of renal hemodynamics as well as effects of an ARB on renal autoregulatory response and tubuloglomerular feedback system have been reviewed previously [1], and will not be discussed in detail here.

2-1-1. RBF—The overall renal hemodynamic responses to ARBs have been variable because of the counteracting influences of the associated decreases in systemic blood pressure [1]. However, if renal perfusion pressure remains within the renal autoregulatory range, RBF is generally increased by an ARB [4, 14]. It is important to note that, rather than predominantly constricting the glomerular efferent arteriole, Ang II elicits vasoconstrictor actions on both pre- and post-glomerular resistance vessels [2]. Therefore, treatment with an ARB dilates both afferent and efferent arterioles under normal conditions [1]. In patients with established glomerular disease, whose afferent arterioles are predominantly dilated to increase intraglomerular pressure and maintain GFR, an Ang II blockade with an ARB would mainly dilate efferent arterioles [1, 15]. Recent studies have also suggested that treatment with an ARB directly dilates the peritubular capillary at the proximal tubules [5, 16] and vasa recta at the medullary thick ascending limb [17].

2-1-2. GFR—Responses of GFR to an ARB have been highly variable [2]. Intrarenal infusion of a subpressor dose of an ARB significantly increased whole kidney GFR in dogs. Most clinical studies have also shown that the GFR remains stable when an ARB is administered. However, a significant reduction in GFR by treatment with an ARB has often been seen in patients with severe renal disease [18, 19]. Decreases in blood pressure by treatment with an ARB are pronounced in a sodium-depleted state [2]. It is common in hypertensive patients with CKD for ARBs to be added to other drugs, including diuretics, under conditions where sodium intake is restricted. It seems likely that ARBs cause marked blood pressure reduction leading to a fall in GFR when extracellular fluid volume is low. Additionally, in patients with established glomerular disease, whose afferent arterioles may be fully dilated to increase intraglomerular pressure and maintain GFR, treatment with an ARB may decrease GFR by predominant vasodilation of efferent arterioles.

2-2. ARB Effects on Renal Cells

2-2-1. Glomerular Cells—Ang II constricts glomerular mesangial cells through AT1 receptor activation, which can be attributed to the reduction in GFR. Therefore, treatment with an ARB may inhibit Ang II-induced mesangial constriction and increase the glomerular filtration coefficient [2]. Mesangial cell proliferation is an important characteristic of many renal injury models, with ARB treatments mitigating or even completely abolishing mesangial cell proliferation in anti-glomerular basement membrane antibody-induced glomerulonephritis [20], Thy-1 nephritis [21], IgA nephropathy [22], anti-thymocyte serum nephritis [23], and diabetic nephropathy [24].

There is increasing evidence indicating that glomerular podocyte (glomerular visceral epithelial cell) abnormalities, including functional changes, loss, and injury, are essentially involved in the progression of albuminuria, glomerular sclerosis, and tubulointerstitial injury [25]. Suzuki *et al* [26] showed that Ang II infusion in rats decreased glomerular podocyte functional molecules in slit diaphragms, such as nephrin and podocin. They also showed that Ang II decreased nephrin expression in cultured podocytes. Ang II-induced reduction in podocyte nephrin and podocin levels was blocked by ARB treatment. Studies have also indicated that podocyte injury is an important characteristic of diabetic nephropathy with ARB treatments mitigating podocyte injury in diabetic animals [13, 27]. Other studies have also suggested that ARB treatments ameliorate high glucose-induced hypertrophy in cultured podocytes [28].

Information regarding ARB effects on parietal epithelial cells is scarce, owing to the difficulty in culturing parietal epithelial cells [29, 30]. Recently, Urushihara *et al* demonstrated that ARB treatments attenuated Ang II-induced cell proliferation and collagen secretion in cultured parietal epithelial cells [31].

2-2-2. Proximal Tubular Cells—Candesartan, an ARB, produced natriuresis/diuresis to a greater degree in obese Zucker rats than in lean Zucker rats. A specific AT2 receptor antagonist, PD123319, after candesartan administration, abolished the natriuretic/diuretic effects of candesartan in obese Zucker rats but not in lean Zucker rats. Infusion of AT2 receptor agonist, CGP-42112A, produced a greater increase in sodium and urine excretion in obese Zucker rats than in lean Zucker rats. These results suggest the upregulation of the AT2 receptors, which plays a role in mediating the natriuretic/diuretic effects of ARB in obese Zucker rats [32]. Using isolated proximal tubules from Sprague-Dawley rats, a further study demonstrated that inhibition of Na+-K+-ATPase activity is involved in this mechanism [33]. Using cultured mouse proximal tubular cells, a study documents AT1-dependent (candesartan-inhibitable) enhancement of bicarbonate reabsorption and AT2-induced (PD123319- and CGP42112A-inhibitable) decrement of bicarbonate absorption. The signaling mechanisms were examined in cultured rabbit proximal tubule cells. The AT2 signaling involves G protein beta- and gamma-mediated phospholipase A2 activation, arachidonic acid release, and downstream events linked to Shc/Grb2/Sos and p21ras rather than protein kinase C [34].

While ARBs were not used, the central role of proximal tubular cells in blood pressure regulation was extensively investigated in a series of elegant studies by Coffman and [35]. To address this important issue, Crowley *et al* [36] employed a kidney cross-transplantation model. Wild-type or AT1 A receptor-deficient mice were transplanted with kidneys from either wild-type or AT1A receptor-deficient donors. The wild-type group contained a full complement of AT1A receptors. Systemic knockout animals expressed AT1A receptors only in the kidney. Kidney knockout animals expressed AT1A receptors in all tissues except the kidney. The total knockout animals completely lacked AT1A receptors. Blood pressure was significantly reduced and to a similar extent in kidney and systemic knockout animals

compared with wild types. There was further reduction in the total knockout mice, suggesting that the mechanisms that lower blood pressure in the kidney and systemic knockout mice were independent.

While this study clearly indicated that AT1A receptors in the kidney play an important role in the pathogenesis of hypertension, the key cellular targets of the renin-Ang system (RAS) that control blood pressure have not been clearly identified. Gurley *et al* [37] demonstrated that RAS actions in the epithelium of the proximal tubule have a critical and non-redundant role in determining the level of blood pressure. They generated mice lacking AT1A receptors only in the renal proximal tubule, and indicated that the abrogation of AT1A receptor signaling in the proximal tubule alone is sufficient to lower blood pressure despite intact vascular responses. Elimination of this pathway reduces proximal fluid reabsorption and alters the expression of key sodium transporters, modifying pressure-natriuresis and providing substantial protection against hypertension. Effectively targeting epithelial functions of the proximal tubule of the kidney should be a useful therapeutic strategy for hypertension.

2-2-3. Distal Tubular Cells—Ang II helps to regulate overall renal tubular reabsorption of salt and water, yet its effects in the distal nephron have not been well studied. Peti-Peterdi *et al* [38] performed studies to determine whether Ang II stimulates luminal sodium transport in the cortical collecting duct. Their results suggest that Ang II directly stimulates epithelial sodium channel activity in the cortical collecting duct. Blocking the AT1 receptor with candesartan or losartan prevented stimulatory effects of Ang II. Regulation of epithelial sodium channel activity by Ang II may play an important role in distal sodium reabsorption.

Beutler *et al* [39] carried out semi-quantitative immunoblotting to identify apical sodium transporter proteins whose levels are regulated by Ang II. In sodium-restricted rats, the AT1 receptor antagonist candesartan markedly decreased the abundance of the alpha subunit of the epithelial sodium channel. This subunit has been shown to be rate-limiting for assembly of mature epithelial sodium channel complexes. Additionally, systemic infusion of Ang II increased alpha epithelial sodium channel protein abundance in the rat kidney cortex. The decrease in levels of alpha epithelial sodium channel protein in response to AT1 receptor blockade was associated with a fall in alpha epithelial sodium channel mRNA levels, consistent with transcriptionally mediated regulation. The effect of AT1 receptor blockade on alpha epithelial sodium channel expression was not blocked by spironolactone, suggesting a direct role of the AT1 receptor in regulation of alpha epithelial sodium channel gene expression. Candesartan administration was also found to increase levels of the beta and gamma subunits. The increase in beta and gamma epithelial sodium channel protein abundance was not associated with a significant increase in the renal abundance of the corresponding mRNAs, suggesting a posttranscriptional mechanism. Immunocytochemistry confirmed the increase in beta and gamma epithelial sodium channel protein abundance and demonstrated candesartan-induced epithelial sodium channel internalization in collecting duct cells. These results support the view that the Ang II receptor regulates epithelial sodium channel abundance, consistent with a role for Ang II in the regulation of the functions of collecting ducts [40, 41].

2-3. ARB Effects on Intrarenal RAS Components

2-3-1. AGT—The only known role of AGT is the sole substrate for renin [42]. Human AGT is 452 amino acids long. However, other species have AGT of varying sizes. The first 12 amino acids are the most important for the activity [43]. AGT is produced constitutively and released into the circulation mainly by the liver [44]. However, the kidneys also synthesize and secrete a considerable amount of AGT [41].

Using *in situ* hybridization, Ingelfinger *et al* [45] demonstrated that the AGT gene was specifically present in proximal tubules. Terada *et al* [46] reported that AGT mRNA was expressed largely in the proximal convoluted tubules and proximal straight tubules, and that there were small amounts in the glomeruli and vasa recta. Richoux *et al* [47] and Darby *et al* [48, 49] showed that the renal AGT protein was specifically located in the proximal convoluted tubules. Kobori *et al* [50] showed that there was strong positive immunostaining for the AGT protein in proximal convoluted tubules and proximal straight tubules, with weak positive staining in the glomeruli and vasa recta. However, there was no staining in distal tubules or collecting ducts. The origin of AGT mRNA and protein in the kidney remains controversial [51-55]. The localization and regulation of intrarenal AGT mRNA and protein are more complicated than they seem [56-60]. Recently, Kamiyama *et al* [61] established a novel method to isolate the three segments of the proximal tubular cells and culture them separately (the S1 segment, the pars convoluta; the S2 segment, the end of the pars convoluta; and the S3 segment, the pars recta). In the near future, these methods may be useful for investigating the segmental regulation of AGT mRNA and protein expression under physiological and pathological conditions.

The feedback loop of the augmented AGT by Ang II has been demonstrated in various tissues, including the liver [62, 63], heart [64, 65], adipose tissue [66, 67], and kidney [68, 69]. Kobori *et al* [50, 51, 70-72] evaluated the effects of Ang II infusions on intrarenal AGT and Ang II levels, and the relationship between urinary excretion of AGT and kidney Ang II and/or AGT levels in several hypertension models. They reported that Ang H-infused rats demonstrated increased abundance of renal AGT mRNA [50] and protein [70], and an enhancement of urinary AGT excretion rates [71]. Chronic Ang II infusion in normal rats significantly increased the urinary excretion rate of AGT in a time- and dose-dependent manner. The urinary excretion rate of AGT closely correlated with systolic blood pressure and kidney Ang II content, but not with plasma Ang II concentration. Urinary protein excretion in volume-dependent hypertensive rats was significantly increased more than in Ang II-dependent hypertensive rats; however, urinary AGT excretion was significantly lower in volume-dependent hypertensive rats than in Ang II-dependent hypertensive rats [51]. Rat AGT was detected in plasma and urine before and after an acute injection of exogenous human AGT. However, human AGT was detected only in the plasma collected after acute administration of human AGT. It was not detected in the urine of Ang II-dependent hypertensive or sham-operated normotensive rats. The failure to detect human AGT in the urine suggests limited glomerular permeability and/or tubular degradation, further suggesting that urinary AGT originates from the kidneys and not from plasma in rats [51, 53]. Chronic ARB treatment offsets the augmented intrarenal/urinary AGT by Ang II infusions in rats [72].

Although generally considered to be characterized by a low activity of circulating RAS, recent studies indicate that treatment with angiotensin converting enzyme (ACE) inhibitors or ARBs reduces cardiac and/or renal dysfunction in Dahl salt-sensitive (DS) hypertensive rats fed a high salt (HS) diet [73-78]. These findings indicate that the local RAS may be inappropriately activated and contribute to the development of hypertension in this animal model. Kobori *et al* [79] reported that DS rats on a HS diet have an inappropriate augmentation of intrarenal AGT that are not reflected in the plasma levels of RAS components. This enhancement may contribute to the impaired sodium excretion that occurs while partaking of an HS diet, and the development of hypertension in this strain.

However, the mechanisms responsible for an inappropriate augmentation of intrarenal AGT were incompletely elucidated in that study. Recent studies report mat an augmented oxidative stress or superoxide anion formation plays an important role in this animal model of hypertension [80-82]. In fact, Kobori *et al* [83] demonstrated that an inappropriate

augmentation of intrarenal AGT during an HS diet is associated with augmented reactive oxygen species in DS rats. In that study, systolic blood pressure was significantly increased by HS challenge, was equally suppressed by a superoxide dismutase mimetic, tempol, and a non-specific vasodilator, hydralazine. An HS diet has been shown to suppress plasma and intrarenal expression of AGT in Sprague-Dawley [84] and Wistar-Kyoto [85] rats. Similarly, in that study, plasma AGT levels were also suppressed by HS. However, kidney AGT levels are enhanced by HS, and tempol treatment prevents this augmentation, while hydralazine does not. This paradoxical enhancement of intrarenal AGT by HS is observed in DS but not in Dahl salt-resistant rats [79]. Thus, these studies provide evidence that an inappropriate augmentation of intrarenal AGT by HS is associated with augmented reactive oxygen species in DS rats. Oxidative stress-induced RAS activation *via* AGT is also supported by a recent structural biology study [86]. The protein AGT must undergo conformational changes to be cleaved into a precursor of the hormone Ang, which increases blood pressure. Oxidative stress seems to mediate this structural alteration [86, 87].

As discussed above, mere is a quantitative relationship between intrarenal/urinary AGT and Ang II production. Urinary AGT excretion rates may provide a useful test in human hypertensive subjects to identify Ang II-dependent hypertension. Recently, AGT enzyme-linked immunosorbent assay systems were established in humans [88], mice, and rats [89]. Using these methods, evidence has been provided in clinical studies indicating that urinary AGT can be a novel biomarker of activated intrarenal RAS in hypertension [90-92], diabetic nephropathy [93-95], and CKDs [96-107].

2-3-2. Renin—Yanagawa *et al* [108] and Moe *et al* [109, 110] showed that renin mRNA and renin-like activity were observed in cultured proximal tubular cells. In addition, low but measurable renin concentrations in proximal tubule fluid have been reported in rats [111].

Renin from juxtaglomerular apparatus cells is released primarily into the interstitium but juxtaglomerular apparatus renin is suppressed in chronic Ang Il-infused hypertensive rats. The source of intratubular renin available that acts on intratubular AGT remains unclear. It is now recognized that renin is also expressed by the principal cells of connecting tubules, and cortical and medullary collecting ducts from rat, mouse and human kidneys [112-114]. In distal nephron segments, renin resides in principal cells co-localizing with aquaporin 2 [113]. Importantly, renin in distal nephron segments is differentially regulated from renin in juxtaglomerular apparatus cells. In response to chronic Ang II infusions, renin mRNA and protein levels in principal cells are augmented, demonstrating a stimulation of distal nephron renin expression during Ang II-dependent hypertension [113]. This effect is an AT1 receptor-mediated process as treatment with an ARB prevents stimulation of distal nephron renin mRNA and protein levels [115, 116]. This is a response distinct from the well-known effect of ARBs that stimulate juxtaglomerular apparatus renin levels. These results indicate that the regulation of renin in principal cells of the collecting duct is different from that of juxtaglomerular apparatus cells, and helps to explain the marked reduction in sodium excretion and impairment in pressure natriuresis that occurs with chronic Ang II infusions [117].

Emerging evidences demonstrate the renoprotective effect of a direct renin inhibitor, aliskiren, in animal [118, 119] and clinical [120] studies. However, it is obvious that to review these papers may be out of focus of this short review article.

2-3-3. ACE/ACE2/Ang 1-7/Mas—With respect to ACE, abundant expression of ACE mRNA [121] and protein [122] were shown in the brush border of proximal tubules of the human kidney. ACE has also been measured in proximal and distal tubular fluid but is more plentiful in proximal tubule fluid [123].

The recent discovery of the Ang II-breakdown enzyme, ACE2, suggests the importance of Ang II degradation in hypertension [124, 125]. Koka *et al* [126] explored the signaling mechanism by which ACE2 is regulated under hypertensive conditions. Quantitative real-time reverse-transcription polymerase chain reaction and immunohistochemistry showed that ACE2 mRNA and protein expression levels were high, whereas ACE expression levels were moderate in both the normal kidney and heart. In contrast, patients with hypertension showed marked ACE up-regulation and ACE2 down-regulation in hypertensive cardiopathy and particularly in hypertensive nephropathy. *In vitro*, Ang II was able to up-regulate ACE and down-regulate ACE2 in human kidney tubular cells, which were blocked by an Ang II receptor antagonist, losartan, but not by the AT2 receptor blocker PD123319. In summary, Ang II is able to up-regulate ACE and down-regulate ACE2 expression levels under hypertensive conditions both *in vivo* and *in vitro*.

To clarify the role of endogenous ACE2 and its cleavage product, Ang 1-7, in the atherogenic stimulation of vascular cells, Hayashi *et al* [127] investigated the effects of pharmacological inhibition of ACE2 and Mas (an Ang 1-7 receptor) on cellular responses against Ang II stimulation. They clearly demonstrated that endogenous ACE2 in vascular cells may contribute to counteracting the Ang II-mediated cellular response partly by upregulating Ang 1-7 signaling through Mas.

2-3-4. AT1/AT2 Receptors—There are two major types of Ang II receptor, AT1 receptor and the type 2 receptor (AT2 receptor). AT2 receptor expression is much lower in adult kidneys compared with AT1 receptor expression [128, 129]. AT1 receptor mRNA has been localized to proximal convoluted and straight tubules, the thick ascending limb of the loop of Henle, cortical and medullar collecting duct cells, glomeruli, arterial vasculature, vasa recta, and juxtaglomerular cells [46]. In rodents, both subtypes of the AT1 receptor (AT1A and AT1B) mRNAs have been demonstrated in the vasculature and glomerulus, and in all nephron segments [129]. The AT1A receptor mRNA is the predominant subtype in nephron segments, whereas the AT1B receptor is more abundant in the glomerulus [130]. Studies using polyclonal and monoclonal antibodies to the AT1 receptor demonstrated that the AT1 receptor protein has been localized on vascular smooth muscle cells throughout the vasculature, including the afferent and efferent arterioles and mesangial cells [131]. AT1 receptors are also present on proximal tubule brush border and baso-lateral membranes, thick ascending limb epithelia, distal tubules, collecting ducts, glomerular podocytes, and macula densa cells [128, 129, 131].

There is no unified view of the status of AT1 receptors in DS rats at this moment in time. Kataoka *et al* [132] reported that AT1 receptor mRNA levels were significantly lower in the glomeruli of DS rats compared with Dahl salt-resistant rats. Strehlow *et al* [133] also demonstrated the down-regulation of AT1 mRNA in the kidneys of DS rats fed a HS diet compared with those fed a low salt diet. They also exhibited that AT1 receptor density is suppressed in the kidneys of DS rats fed a HS diet compared with those on a low salt diet. Nishiyama *et al* [134] presented that intrarenal AT1 receptor protein levels were similar in DS rats that were either fed an HS or low salt diet. Harrison-Bernard *et al* [135] also reported that intrarenal AT1 receptor protein levels were unaltered between DS rats and Dahl salt-resistant rats fed a HS diet. However, Ruiz-Opazo *et al* [136] demonstrated that DS rats have some mutations within their genes, leading to increased receptor affinity for Ang II.

These controversial observations may come from different substrains of Dahl rats, different sampling time points, or a combination of both these factors.

ARBs are a specific new class of non-peptide Ang II receptor antagonists. They work by blocking the AT1 receptor in tissues. Several factors including the level of Ang II and the number of AT1 receptors available influence the action of ARBs. The number of AT1 receptors on the cell surface determines the magnitude of the blocker's effect. Sodium depletion has been associated with increases in the numbers of AT1 receptors and increased responses to ARB. By binding to the AT1 receptor, an ARB decreases aldosterone, vasopressin, and catecholamine release [137-142]. ARB also causes vascular vasodilation, and inhibition of sodium and water reabsorption in the kidney. Collectively, these effects lead to a reduction in blood pressure [143].

AT1 receptors are also localized in juxtaglomerular cells. Inhibition of AT1 receptors is associated with an increase in renin secretion that leads to further Ang II formation. Accumulation of Ang II as a result of ARB treatment can theoretically compete with ARBs at the receptor binding site and diminish therapeutic efficiency of the treatment. However this issue remains controversial. The increase in Ang II after ARB treatment allows stimulation of the AT2 receptor. Activation of the AT2 receptor is associated with increased tissue release of nitric oxide, guanylate cyclase, and tissue bradykinin [144]. In contrast to the AT1 receptor, the AT2 receptor has antigrowth properties and stimulates programmed cell death. Thus, the AT2 receptor seems to counterbalance the effects of the AT1 receptor.

Taking into account that ACE inhibitors do not completely inhibit Ang II formation, and that ARBs lead to Ang II accumulation with a possible underlying “escape mechanism,” should ACE inhibitors and ARBs be used together? Ang II, generated by non-ACE mechanisms, could be inhibited at the receptor level by ARBs. While inhibition of the RAS *via* the AT1 receptor could be strengthened, nitric oxide-dependent vasodilator pathways activated by ACE inhibitors remain intact. AT2 receptor-mediated actions could be activated by the shifting of RAS to the AT2 receptor. Therefore, the combinadon therapy of ACE inhibitors and ARBs would be more beneficial than the monotherapy by ACE inhibitor or ARB.

Some trial studies have provided evidence of this [145-148]. The Randomized Evaluation of Strategies for Left Ventricular Dysfunction study revealed that a combination of an ACE inhibitor and an ARB decreased blood pressure and improved the ejection fraction to a greater extent than treatment with either drug alone in patients with congestive heart failure [145]. The Valsartan in Heart Failure Trial demonstrated that a combination of an ACE inhibitor and an ARB reduced hospitalization for heart failure in patients with congestive heart failure by 30%. However, there was no decrease in all-cause mortality observed [146].

3. Mechanisms Underlying the Renoprotective Effects of an ARB in Type 2 Diabetic Nephropathy

In patients with type 2 diabetic nephropathy, microalbuminuria is an independent risk for cardiovascular events [8]. The anti-albuminuric effects of ARBs are beyond their blood lowering and hemodynamic effects [6, 7]. Furthermore, long-term observational studies have demonstrated that remission of nephrotic-range albuminuria is associated with substantial reductions in the risks of progressing to ESRD and cardiovascular events, greatly improving the survival rate of type 2 diabetic patients [149]. Based on this accumulating clinical evidence, current national guidelines have recommended the use of RAS inhibitors, including ARBs, as a first-line anti-hypertensive drug for hypertensive patients with type 2 diabetic nephropathy [9-11]. In this section, we briefly discuss the mechanisms responsible for renoprotective effects of an ARB during both early and late phases of type 2 diabetic nephropathy.

3-1. Early Phase (Microalbuminuria)

3-1-1. Clinical Observations—There is substantial clinical evidence that ARBs are effective in reducing urinary albumin excretion rate, independent of blood pressure reduction in type 2 diabetic nephropathy with microalbuminuria [150, 151]. Therefore, treatment with ARBs is now standard practice in the management of early type 2 diabetic nephropathy in normotensive and hypertensive patients with microalbuminuria. Makino *et al* [150] showed that treatment with an ARB, telmisartan, dose-dependently elicits a regression to normoalbuminuria in type 2 diabetic nephropathy patients with microalbuminuria. Similarly, a recent meta-analysis by Blacklock *et al* [152] showed that high-dose compared with low-dose ARBs reduced urinary albumin excretion rates and increased regression to normoalbuminuria in type 2 diabetic nephropathy patients with microalbuminuria. Interestingly, the authors also showed that adverse events seem to be more frequent in the high-dose ARB-treated groups, but these differences were not statistically significant [152]. Other studies have also demonstrated that treatment with ARBs prevent progression to macroalbuminuria in patients with diabetes and microalbuminuria [153].

Recently, the Randomized Olmesartan and Diabetes Microalbuminuria Prevention (ROADMAP) study was conducted to examine if an ARB, olmesartan, can prevent the onset of microalbuminuria [147]. The ROADMAP study was a placebo-controlled, multi-center, double-blind, parallel-group study investigating the effects of olmesartan on the incidence of microalbuminuria in 4,400 patients with type 2 diabetes and normoalbuminuria. The results showed that early treatment with olmesartan significantly reduced the occurrence rate of microalbuminuria in type 2 diabetes patients. These data suggest that early treatment with an ARB decreases future risk of cardiovascular events as well as progressing to ESRD in patients with type 2 diabetes and normoalbuminuria. The ROADMAP study was not able to address these issues because of a limitation in the observational period. There has been a report indicating that the anti-microalbuminuric effects of ARBs are not seen in all hypertensive patients. A recent meta-analysis by Daien *et al* [154] showed that prevention of renal dysfunction is not significantly different with RAS inhibitors when compared with other antihypertensive agents in patients with essential hypertension and no pre-existent renal disease. Importantly, this meta-analysis did not include the studies that specifically enrolled only patients with diabetes. Thus, it can be speculated that the preventive effects of ARBs on microalbuminuria and the following renal injury may be restricted to type 2 diabetic nephropathy.

3-1-2. Basic Studies—We have previously shown that initiation and progression of microalbuminuria is associated with augmented intrarenal Ang II levels and podocyte abnormalities. This occurs before morphological injuries to renal tissues become apparent in type 2 diabetic Otsuka Long-Evans Tokushima fatty (OLETF) rats [151]. These rats exhibit pathological features of renal injury similar to those seen in human type 2 diabetes patients [155]. Furthermore, strict Ang II blockade with telmisartan from an early stage can prevent progression from microalbuminuria to macroalbuminuria independently of its effects on blood pressure and glucose metabolism in OLETF rats. These effects of telmisartan were associated with suppression of the augmentation of intrarenal Ang II levels and podocyte abnormality [151].

Additional studies were performed to determine if early treatment with olmesartan prevented the onset of microalbuminuria by attenuating glomerular podocyte injury in obese type 2 diabetic OLETF rats [156]. We found that podocyte injury occurs in the juxtamedullary glomeruli prior to superficial glomeruli in type 2 diabetic rats with microalbuminuria. These data indicate that the initiation of microalbuminuria is accompanied by juxtamedullary glomerular podocyte injury in type 2 diabetes. We also showed that treatment with

olmesartan from the pre-albuminuria stage can prevent an increase in intrarenal Ang II levels, along with the onset of microalbuminuria [156]. These findings support the observations of the ROADMAP studies [147] and the concept that selective inhibition of Ang II during the early stages of type 2 diabetes can prevent the onset of diabetic nephropathy.

3-2. Late Phase (Overt Albuminuria)

3-2-1. Clinical Observations—In patients with type 2 diabetes and overt albuminuria, the degree of albuminuria is closely correlated to renal and cardiovascular outcome [8, 157] as well as survival time [158]. Reduction in urinary albumin excretion rate can be achieved through early and aggressive treatment with antihypertensive agents. In particular, ARBs are effective at reducing urinary albumin excretion rates, in addition to their blood pressure-lowering effects in type 2 diabetic patients with overt albuminuria [6, 7]. Because remission of nephrotic-range albuminuria is associated with a substantial reduction in the risks of progressing to ESRD and cardiovascular events, and in gready improving the survival rate of type 2 diabetic patients [149], treatment with ARBs is standard practice in the management of early type 2 diabetic nephropathy with overt albuminuria. However, to the best of our knowledge there has been no study to demonstrate that reduction in albuminuria by an ARB actually leads to substantial reductions in the risks of progressing to ESRD and cardiovascular events, and in the end improves the survival rate of type 2 diabetic nephropathy patients with overt albuminuria.

The Olmesartan Reducing Incidence of End Stage Renal Disease in Diabetic Nephropathy Trial (ORIENT) study was a randomized, double-blind, placebo-controlled study to examine the effects of olmesartan medoxomil on renal and cardiovascular risks in type 2 diabetic patients with overt proteinuria [148]. Patients treated with antihypertensive therapy (73.5% received a concomitant ACE inhibitor) were given either olmesartan or placebo once per day. Primary composite outcomes include doubling of serum creatinine, end stage renal disease and death. Data showed that olmesartan significantly decreased blood pressure, proteinuria and rates of change of reciprocal serum creatinine. Olmesartan reduced composite cardiovascular outcomes by 34%, independent of the effects of ACE inhibitor treatment after adjustment for blood pressure and other factors [148]. However, composite renal outcome was not significantly improved by the addition of olmesartan. These data support the hypothesis that, in patients with type 2 diabetes and overt albuminuria who have been treated with an ACE inhibitor, reduction in urinary albumin excretion rate by additional treatment with an ARB elicits substantial reduction in the risks of cardiovascular events. Nevertheless, the mechanisms by which treatment with an ARB leads to regression of overt albuminuria have not been clarified by the ORIENT study.

3-2-2. Basic Studies—Studies were performed to determine whether glomerular podocytes were damaged in a heterogeneous manner depending on their location, during the progression of overt albuminuria in type 2 diabetic OLETF rats [159]. We observed that juxtamedullary glomerular podocyte injury reaches a severe condition more rapidly than superficial glomerular podocyte injury in type 2 diabetic rats with overt albuminuria. Furthermore, treatment with olmesartan resulted in a marked reduction in albuminuria, with a regression of superficial glomerular podocyte injury. Severely damaged juxtamedullary glomerular podocyte was not affected by olmesartan [159]. These data indicate that remission and/or regression of nephrotic-range albuminuria induced by treatment with ARBs is accompanied by the regression of superficial glomerular podocyte injury in type 2 diabetes, but that regression of advanced juxtamedullary glomerular podocyte injury may be hard to achieve.

4. Conclusion

In this review, we have briefly summarized our current understanding of the pharmacological effects of ARBs in the kidney. Although we do not discuss the issues related to Ang II-independent pharmacological effects of an ARB, the mechanisms responsible for the renoprotective effects of ARBs are multiple and complex as depicted in (Fig. 1). The adequate use of ARBs with true understanding of the pharmacological mechanisms behind their renoprotective effects may lead us to new treatments for patients with CKD.

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Abbreviations

ACE	Angiotensin converting enzyme
AGT	Angiotensinogen
Ang	Angiotensin
ARB	Angiotensin II type 1 receptor blocker
AT	Angiotensin II type
CKD	Chronic kidney disease
DS	Dahl salt-sensitive
ESRD	End-stage renal disease
GFR	Glomerular filtration rate
HS	High salt
OLETF	Otsuka Long-Evans Tokushima fatty
RAS	Renin-angiotensin system
RBF	Renal blood flow

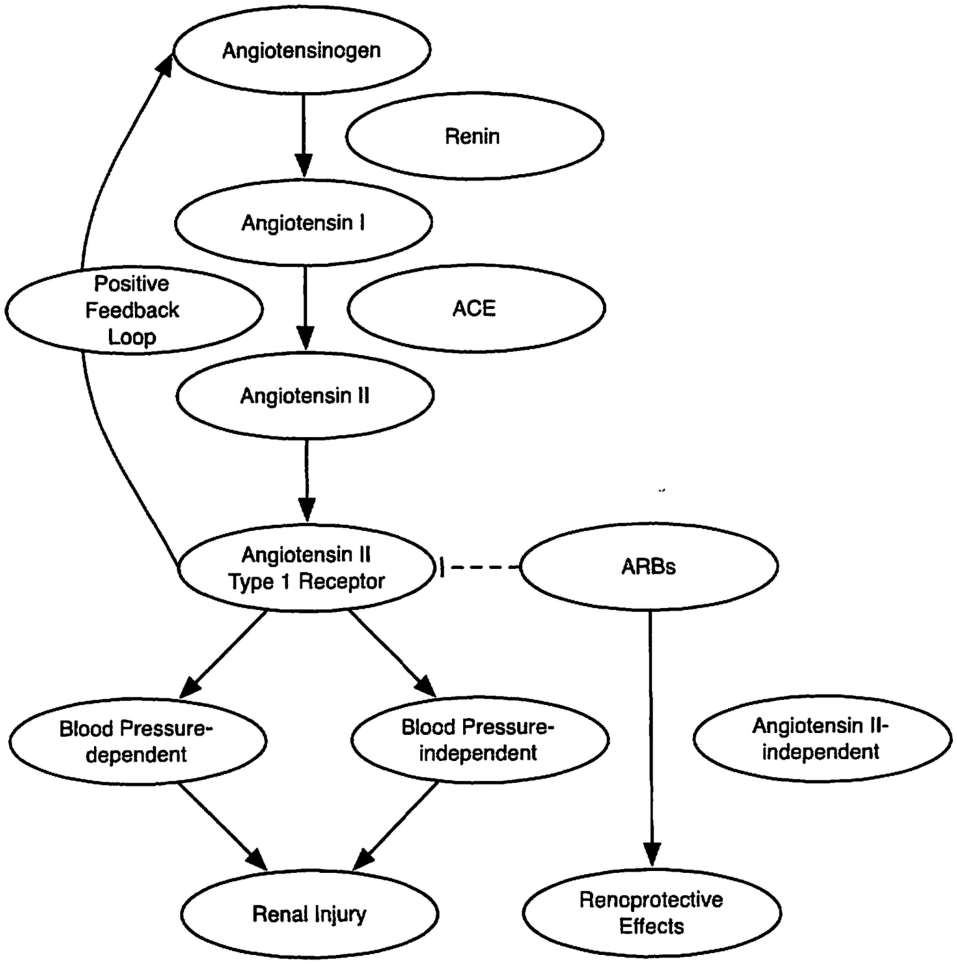

Fig. 1.
Cascade of the RAS and the renoprotective effects of ARBs.