Chapter 33
Antifibrotic Roles of RAAS Blockers: Update

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Abstract The rennin–angiotensin–aldosterone system (RAAS) has been well documented in regulating blood pressure, fluid volume, and sodium balance. Overactivity of RAAS promotes both systemic and regional glomerular capillary hypertension, which could induce hemodynamic injury to the glomerulus, leading to kidney damage and renal fibrosis via profibrotic and proinflammatory pathway. Therefore, the use of RAAS inhibitors (i.e., ACEIs, ARBs, and MRAs) as the optional therapy has been demonstrated to prevent proteinuria, and kidney fibrosis and slow the decline of renal function effectively in the process of kidney disease during the last few decades. Recently, several new components of the RAAS have been discovered, including ACE2 and the corresponding ACE2/Ang (1-7)/Mas axis, which are also present in the kidney. Besides the classic RAAS inhibitors target the angiotensin-AT1-aldosterone axis, with the expanding knowledge about RAAS, a number of potential therapeutic targets in this system is emerging. Newer agents that are more specific are being developed. The present chapter outlines the insights of the RAAS agents (classic RAAS antagonists/the new RAAS drugs), and discusses its clinical application in the combat of renal fibrosis.

Keywords Renin–angiotensin–aldosterone system (RAAS) · Fibrosis · Antagonists

33.1 Introduction

Renal fibrosis is a common step in the progression of a variety of chronic kidney diseases to end-stage renal disease. It is characterized by excessive accumulation of extracellular matrix, representing the final target to treat chronic kidney disease (CKD). It is widely accepted that the degree of renal fibrosis correlates well with kidney function and CKD stage (Schainuck et al. 1970).

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The rennin–angiotensin–aldosterone system (RAAS) plays a key role in regulating blood pressure, fluid volume, and sodium balance. Overactivity of the RAAS is involved in the pathology progression of a variety of diseases, such as hypertension, atherosclerosis, left ventricular hypertrophy, myocardial infarction, and heart failure. Researchers have demonstrated that the overactivity of RAAS contributed to the progression of renal fibrosis and that RAAS antagonists prevented renal fibrosis and slowed the decline in renal function in patients with kidney disease.

In 1971, Oparil, S et al. described the main cascade of the RAAS system (Oparil and Haber 1971). Plasma angiotensinogen is cleaved by renal renin, generating angiotensin I (AngI), which is then converted to angiotensin II (AngII) by endothelial angiotensin-converting enzyme (ACE). AngII is considered the most important RAAS peptide and is associated with vasoconstriction and high blood pressure. AngII binds to the type–1 AngII receptor (AT1) in a variety of tissues. Then, aldosterone is stimulated via the AT1 receptor in the adrenal gland, facilitating sodium retention by the kidney when aldosterone binds to the mineralocorticoid receptor. More recently, several new components of the RAAS have been discovered, including ACE2 and the corresponding ACE2/Ang (1-7)/Mas axis, which are also present in the kidney.

The classic RAAS inhibitors target the angiotensin-AT1-aldosterone axis. However, with the expanding knowledge about RAAS, the number of potential therapeutic targets in this system is increasing. In this secession, we discuss novel agonists and antagonists of the RAAS that might combat renal fibrosis (Fig. 33.1).
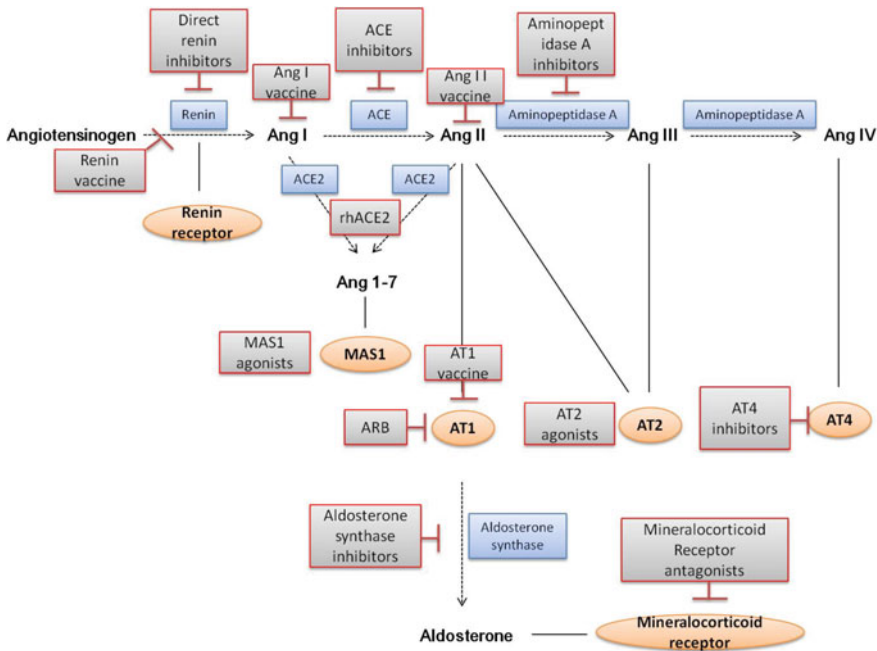

Fig. 33.1 Antifibrotic role of RAAS blockers in renal fibrosis

Multiple drugs have been well established to interfere with RAAS at different levels, such as renin inhibitors, ACE inhibitors, ARBs, and mineralocorticoid receptor antagonists, which directly inhibits renin, ACE, AT1R, and the mineralocorticoid receptor, respectively. Novel blockers are developed to target Aminopeptidase A, the enzyme that catalyzes the conversion of Ang II to Ang III, and Ang III to Ang IV. On the other hand, replenishment of RhACE2 are used to activate ACE2, the enzyme that catalyzes the conversion of Ang I and Ang II to Ang (1-7). Moreover, novel agonists have been designed to target AT2, AT4 and MAS1 receptors. In addition to inhibitors and agonists, alternative strategies such as vaccines specifically target rennin, AngI, AngII, and AT1 receptor have also been developed. ACE: angiotensin-converting enzyme; Ang: angiotensin; ARB: angiotensin receptor blocker; AT1: type-1 Ang II receptor; AT2: type-2 Ang II receptor; AT4: type-1 Ang II receptor; MAS1: proto-oncogene Mas; rh: recombinant human.

33.2   Classic RAAS Antagonists

33.2.1   Angiotensin-Converting Enzyme Inhibitors (ACEIs)

Stopping the activation of AT1 is an attractive antifibrosis target in RAAS. ACE inhibitors (ACEIs) block the synthesis of AngII, which is catalyzed by ACE, preventing the conversion of AngI to AngII, limiting the effect of AngII and further decreasing the secretion of aldosterone and vasopressin.

The effectiveness of ACEIs in preventing or attenuating kidney disease in the clinic may be partly due to hemodynamics and non-hemodynamic factors. ACEIs can reduce the intraglomerular pressure by reducing the afferent arterial pressure and slow the breakdown of bradykinin, decreasing the size and charge selectivity of the glomerular cell wall. In addition, ACEIs can reduce cytokine production, such as by transforming growth factor-beta (TGF-β), which induces glomerulosclerosis and renal fibrosis (Zhang et al. 2017).

Enalapril, an ACEI, significantly attenuated BSA-induced rat renal tubule-interstitial inflammation and fibrosis by suppressing NLRP3 inflammasome expression (Ding et al. 2014). Furthermore, in a UUO mouse model, the amelioration of Enalapril on renal fibrosis was mast cell-dependent, as there was no effect of Enalapril on mast cell-deficient mice developing renal fibrosis (Sun et al. 2016).

In 1993, the CAPTOPRIL trial studied the effect of ACEI captopril on people with type 1 diabetes with proteinuria and showed that, compared with the placebo, treatment with captopril led to a 30% reduction in proteinuria, a 43% reduction in the risk of the primary end point of doubling of serum creatinine and a 50% reduction in the combined end point of death and the need for dialysis or renal transplantation, depending on BP levels (Lewis et al. 1993). In the Bergamo Nephrology Diabetic Complications trial, 1204 type 2 diabetic patients with hypertension and normal urine albumin excretion were studied and followed for a median of 3.6 years. The results

showed that trandolapril decreased the incidence of persistent micro-albuminuria compared to placebo and verapamil, indicating that ACEIs not only could delay the progression of diabetic nephropathy but also were able to prevent the onset of nephropathy (Ruggenenti et al. 2004).

Although ACEIs result in lower levels of AngII in the blood, AngII produced by alternative conversion pathways can still be combined with AT1. When AngII levels decline, AngII accumulates by non-ACE pathways, which is called "ACE escape." The phenomenon may be due to the increase in renin release reactively because of AngII loss. ACEIs could also upregulate Ang1-7 and bradykinin, which may be related to target organ protection by ACEIs (Sureshkumar 2008).

33.2.2   Angiotensin Receptor Blockers (ARBs)

Angiotensin receptor blockers (ARBs), which are highly selective for the AT1 receptor, increase AngII levels in circulation and retard AngII binding to the AT1R. ARBs have similar effects to those of ACEIs, including regulating blood pressure and maintaining endothelial function; however, they do not increase bradykinin production. In addition, there are no such ACE escape problems In addition, because the blockade of AT1R AngII binds with AT2R alternatively or is diverted to Ang1-7, ARBs exert beneficial effects.

Obata et al. concluded that in hypertensive glomerulosclerosis animal models, RAAS activation and sensitivity to AngII increase in glomeruli (Obata et al. 1997). In rats treated with candesartan for 12 weeks, UAE decreased, as did TGF-β, fibronectin (FN), and RAAS components.

Wang et al. (2013) investigated the therapeutic role of ARB in an IgA nephropathy rat model and found that administration of losartan decreased urinary protein levels and reduced serum BUN, Scr, TGF-β1, FN, α-SMA, and FGF-1, finally delaying the progression of advanced IgA nephropathy with impaired renal function. In the IDTN and RENAAL trials, ARB treatment reduced the relative risk of reaching the primary composite end point—death, doubling of serum creatinine, or end-stage renal disease—in people with type 2 diabetes with kidney involvement by 20 and 16%, respectively (Lewis et al. 2001; Brenner et al. 2001).

33.2.3   Mineralocorticoid Receptor Antagonists (MRAs)

Aldosterone is a mineralocorticoid hormone that promotes sodium retention and renal fibrosis by inducing renal vasoconstriction, oxidative stress, and inflammation (Bertocchio et al. 2011). MRAs are still mainly used as diuretics in the treatment of hypertension but also heart failure, with a significant reduction in mortality (Pitt et al. 1999). In CKD patients treated with an ACEI or ARBs, increased aldosterone levels were observed, called the "aldosterone escape phenomenon," and activated

the mineralocorticoid receptor (MR) (Lijnen et al. 1982). This supported the use of mineralocorticoid receptor antagonists (MRAs) in addition to ACEI or ARBs on renal fibrosis.

MRAs are classified as conventional steroidal and nonsteroidal compounds. Steroidal compounds (spironolactone and eplerenone) inhibit the effects of aldosterone by competing for its ligand-binding domain on MR and prevent MR from adopting the active conformation.

Researchers demonstrated that administration of spironolactone to the STZ-induced diabetic rat reduced proteinuria and decreased the expression of collagen I/IV, TGF-β, and attenuated glomerular and tubulo-interstitial fibrosis, despite the absence of BP or blood glucose reduction (Kolkhof et al. 2015). In addition, spironolactone significantly alleviated cisplatin-induced nephrotoxicity and renal fibrosis both in vivo and in vitro (Elseweidy et al. 2018).

It was reported that inhibition of local aldosterone by eplerenone prevented the upregulation of transforming growth factor-β1, connective tissue growth factor, plasminogen activator inhibitor type 1, and collagen I in adrenalectomized rats (Sun et al. 2015).

33.2.4 Direct Renin Inhibitors (DRIs)

Many patients have elevated AngII levels due to AngII reactivation under ACE inhibition and ALDO escape during long-term treatment with an ACEI or an ARB. A similar phenomenon has been discovered for MRAs, called "aldosterone escape." The patients who take MRAs may have higher aldosterone compared to pretreatment. Direct renin inhibitors block the RAAS at an earlier stage in the cascade than do ACEIs and ARBs and prevent the formation of both AngI and AngII by both ACE and non-ACE pathways (Nadeem and Batisky 2014).

The development of renin inhibitors has provided an opportunity to evaluate the effects of direct renin inhibition (DRIs) as another means of RAAS blockade. Aliskiren is the first in a new class of orally effective DRIs approved for the treatment of hypertension (Jensen et al. 2008).

The safety and efficacy of aliskiren have been well defined through preclinical pharmacological safety studies. These were also assessed in adult patients with varying degrees of renal or hepatic impairment (Kelly et al. 2007). The results indicate that there is no need to adjust the dose of aliskiren in patients with renal or hepatic impairment; however, according to the package insert, it is recommended to exercise caution when prescribing aliskiren to patients who have moderate to severe renal dysfunction, a history of dialysis therapy, nephrotic syndrome, or renovascular hypertension due to the scarcity of data in these patients and the potential for other agents affecting the RAAS to increase serum creatinine and blood urea nitrogen levels (Miyata et al. 2014).

Several clinical studies have demonstrated that aliskiren is effective on CKD (Li et al. 2012; Miyata et al. 2014). Persson et al. reported that aliskiren (300 mg daily)

treatment reduced UACR, with a 17% reduction by days 2–4, a 31% reduction by days 8–10, and a maximum reduction of 44% at the end of the treatment (day 28) (Persson et al. 2008). Wu et al. found that aliskiren at a dosage of 150 mg/day for six months in 103 Chinese CKD patients (both with and without diabetes) had a favorable effect on reducing residual proteinuria and inadequately controlled blood pressure (Wu et al. 2014). Mechanically, aliskiren prevents renal disease progression by suppressing both angiotensin I and II in RAAS-activated pathology; moreover, Renke revealed that aliskiren also attenuated oxidative stress and improved the functional status of tubules in nondiabetic nephropathy (Renke et al. 2014). Collectedly, these results strongly suggest that aliskiren has beneficial effects for renoprotection with or without BP-lowering effects in CKD patients. In addition, aliskiren can reduce sympathetic hyperactivity, which is often exhibited and contributes to the pathogenesis of hypertension and CVD in patients with CKD (Miyata et al. 2014).

DRIs, in theory, seem to have the same adverse effects as do other RAAS blockers. A pooled analysis showed that the most common adverse events thought to be related to aliskiren treatment were headache, diarrhea, nasopharyngitis, back pain, and dizziness. Hyperkalemia, which is a frequent concern in CKD patients, is the primary danger of RAAS-blocking medications. The blockade of the RAAS leads to a decrease in aldosterone levels. Because of the urinary potassium excretion caused by aldosterone, RAAS blockers can cause potassium retention. Several clinical trials targeting aliskiren in CKD patients with tracked potassium levels showed that there was no significant trend for increased hyperkalemia by aliskiren in patients with CKD in these trials (Vaidyanathan et al. 2007).

Currently, many controversies regarding the use of aliskiren in diabetic kidney disease are under discussion. Numerous animal models of DKD have shown significantly improved BP control and proteinuria with the use of aliskiren. At the level of the glomerulus, the use of aliskiren is associated with decreased podocytopathy and glomerular pressure. Tubulo-interstitial fibrosis and levels of profibrotic and oxidative markers were found to be reduced with aliskiren. The AVOID trial showed that aliskiren might have additional renoprotective effects that are independent of its blood pressure-lowering effects in patients with hypertension, type 2 diabetes and nephropathy when added to ARB. Unfortunately, another clinical trial, the ALTITUDE trial, due to a lack of apparent benefit and a higher risk of side effects, was prematurely stopped for futility in patients with type 2 diabetes and micro-albuminuria, macro-albuminuria, or cardiovascular disease. Therefore, the FDA has given a formal recommendation about aliskiren: it should not be used in association with ARBs or ACEIs as dual therapy in patients with diabetes mellitus or renal disease (Parving et al., 2012).

A new drug, DRI-ACT-077825, was proved to be safe in the human body when administered once daily in 2013. ACT-077825 inhibited plasma renin activity, while the immunoreactive renin level increased. However, the decrease in plasma renin activity is short lived and is not affected by the dose within 7 days. The effect of DRI on blood pressure is controversial. More research is needed to identify inconsistencies (Nicolas et al. 2013).

Thus, aliskiren has beneficial effects for renoprotection, the control of BP, and the prevention of CVD in patients with CKD. However, the possible adverse effects and potential interactions with other drugs being used together have to be carefully considered. In DKD patients, it clearly has an antihypertensive effect and a proteinuria-reducing effect that are greater than placebo. These benefits are not superior to those offered by currently used ACEIs or ARBs. Thus, DRI is a reasonable alternative to the use of ACEI or ARB in DKD, especially in settings where ACEI or ARB may not be used. Another limitation is that there is still a lack of high-quality studies to confirm the effects of aliskiren in patients with CKD.

33.3 New RAAS Drugs of the AngII-AT1/2R Axis

33.3.1 The Angiotensin II Subtype-2 Receptor (AT2R) Agonist

AngII primarily activates two receptor subtypes, AT1R and AT2R. It is well established that activation of AT1R by AngII mediates pathophysiological effects such as vasoconstriction, proliferation, fibrosis, oxidative stress, and inflammation, which occur in multiple organs. On the other hand, activation of AT2R is thought to counter-regulate the pathophysiological effects induced by AT1R and exert vasodilator, antifibrotic, antiproliferative, and anti-inflammatory effects, as well as natriuretic and antihypertensive effects, in renal disease (Wang et al. 2017; Hallberg et al. 2018).

In contrast to AT1R, AT2R is very sparsely expressed and is abundant only in certain tissues, such as the vascular endothelium, kidney, and brain. In the kidney, AT2R is mainly localized to renal vessels, glomeruli, and tubules. AT2R activation also opposes the vasoconstrictor actions of AT1R by promoting dilation of the afferent and efferent arterioles. A previous study revealed that the appropriate balance between AT1R and AT2R activation might therefore play a key role in regulating the physiological functions of the renal systems (Ozono et al. 1997). Mechanistically, the following phenomena might contribute to the renoprotection of AT2R. First, AT2R stimulates mitogen-activated protein kinase phosphatase-1 (MKP-1) and inhibits ERK activity, resulting in the reduction in mitogen-activated protein kinase (MAPK) activity and growth inhibition. Second, AT2R regulates the production of renal nitric oxide (NO), guanosine cyclic 3′,5′-monophosphate (cGMP), and bradykinin (Siragy and Carey 1997; Abadir et al. 2003; Matavelli and Siragy 2015), indicating that it could be involved in the induction of vasodilation. In addition, Rompe et al. (2010) showed that targeting AT2R exerted a direct anti-inflammatory effect by inhibiting NF-κB activation, leading to reduced TNF-α-mediated IL-6 release from fibroblasts. Together, these data demonstrate that AT2R plays a substantial role in organ inflammation and tissue fibrosis and that its cellular signaling pathways go in opposite directions to those of AT1R.

In 2004, a novel AT2R agonist named Compound 21 (C21) was developed. C21 is a nonpeptidic compound that is orally and systemically active with an oral bioavailability of 20–30%. C21 is a highly selective AT2R agonist that is in the final stage of a preclinical trial (Steckelings et al. 2012). Many basic experiments have revealed that C21 can inhibit inflammatory responses and renal fibrosis via activation of AT2R. It lacks AT1R affinity and was demonstrated in human embryonic kidney cells to have 4000-fold selectivity to AT2R (Bosnyak et al. 2011). If clinical studies confirm its efficacy, this compound could be useful for the management of diverse cardiovascular and kidney diseases, including diabetic kidney disease and glomerulonephritis (Pandey and Gaikwad 2017).

More recently, additional AT2R ligands have been investigated in the clinic. Namely, MP-157 is an AT2R agonist in phase I clinical trials in Europe, aimed at the cardiovascular system, according to information from Mitsubishi Tanabe Pharma "State of New Product Development (as of August 2, 2016)." Unfortunately, its structural formula is still not disclosed. Another AT2R antagonist, EMA401, from Spinifex Pharmaceuticals Pty Ltd, Australia, and now acquired by Novartis, has been successfully tested in a phase II trial in patients with neuropathic pain (Rice et al. 2014). Thus, it is conceivable that AT2R agonists will play an important role in the future.

33.3.2   Vaccines Against the RAAS

The concept of immunization as a treatment for hypertension is not new, and several studies have yielded insights into an angiotensin vaccine as a strategy for inhibiting RAAS. As a specific target antigen, the antihypertension vaccine is complicated by the multifactorial etiology of hypertension compared to the vaccines generated against bacteria and viruses (Do et al. 2010). RAAS is potentially the most important regulator of systemic blood pressure and is believed to be a major factor in hypertension onset. Antibodies against renin, angiotensinogen, AngI, AngII, ACE, and AT1/2 have been studied in experiments in an attempt to create a vaccine that would chronically suppress RAAS activity.

The renin vaccine is the earliest vaccine that effectively reduces blood pressure in animal models; its binding to renin inhibits the interaction between renin and angiotensinogen and suppresses renin's enzymatic activity. Because the structure of renin is highly species specific, vaccination against heterologous renins led to the production of antibodies that produced only incomplete suppression of renin's enzymatic activity. Subsequent advances in human renin purification made it possible to actively immunize marmosets against human renin. Unfortunately, these experiments also revealed that the renin vaccine led to the onset of various autoimmune diseases that emerged as a major concern for any immunotherapy directed at endogenous RAAS components (Gradman and Pinto 2008).

Unlike renin, AngI and AngII are very small peptide molecules composed of ten and eight amino acids, respectively. It is well documented that smaller

molecules will be less likely to stimulate autoimmune diseases. In animal models, AngI vaccines between two carriers, tetanus toxoid (TT) (PMD-2850) and keyhole limpet hemocyanin (KLH) (PMD3117), induced equivalent immune responses and inhibition of the pressor effects of exogenous AngI. In a phase IIa clinical trial, three or four injections of the angiotensin I vaccine PMD3117 resulted in antibody production with titers peaking 64 days after protocol initiation and no evidence of autoimmune disease. The results revealed that PMD3117 could block the RAAS system. Therefore, a new formulation of PMD3117 has been developed. Confirmation of safety is the priority, and renoprotection should be addressed by further clinical assessment in phase IIb and phase III trials (Gardiner et al. 2000; Downham et al. 2003; Do et al. 2010).

The AngII vaccine is a logical target for immunotherapy. In 2007, Cytos Biotechnology developed an AngII-specific vaccine (CYT006-AngQb) composed of an AngII peptide with an N-terminal Cys-Gly-Gly extension that is covalently coupled to virus-like particles (VLP) derived from the coat protein of the bacteriophage Qb. Ambühl PM showed that VLP (AngQb) reduces blood pressure in SHR to levels obtained with an ACE inhibitor and is well tolerated in humans. Therefore, vaccination against angiotensin II has the potential to become a useful antihypertensive treatment that provides long-lasting effects and improves patient compliance (Ambuhl et al. 2007). Some studies reported that SHR exhibited an SBP decrease of 17 mmHg after immunization with a peptide-based vaccine made of a seven-amino acid sequence (AFHYESR) from the second extracellular loop of rat AT-1A receptor (ATR12181), which alleviated cardiac hypertrophy and attenuation of kidney injuries. There were no signs of autoimmune diseases in the biopsied sections of kidney. Because Freund's adjuvant is not safe for use in humans, the next phase of the study will focus on the use of ATR12181 in combination with VLP in human subjects (Zhu et al. 2006).

In conclusion, vaccination to achieve chronic RAAS suppression represents a novel approach to treating hypertension. However, the effect of vaccines on renal fibrosis after controlling hypertension and blocking the RAAS system is still unknown.

33.4   New Drugs of the Ang1-7-Mas Axis

33.4.1   Human Recombinant ACE2 (hrACE2)

In the kidneys, ACE2 is expressed in the proximal tubules and less strongly in the glomeruli. The synthesis of inactive Ang 1-9 from AngI and the catabolism of AngII to produce Ang 1-7 are the main functions of ACE2 (Nishiyama et al. 2002; Tikellis et al. 2004). There may be two mechanisms to explain the beneficial effect of ACE2 in vascular diseases. First, ACE2 reduced the AngII interaction with AT1R by inducing AngII degradation; second, ACE2 reduced vasoconstric-

tion, water retention, and reactive oxygen stress by increasing Ang1-7 synthesis (Clarke and Turner 2012). Zhong et al. found that daily treatment with recombinant human ACE2 (hrACE2) reduced the AngII-induced pressor response and normalized renal AngII levels and oxidative stress, which indicated that human ACE2 prevented AngII-mediated renal oxidative stress, inflammation, and tubulo-interstitial fibrosis in animal models (Zhong et al. 2011). Oudit GY found that treatment with hrACE2 attenuated diabetic kidney injury in the Akita mouse in association with a reduction in blood pressure and a decrease in NADPH oxidase activity (Oudit et al. 2010). Furthermore, accumulating evidence indicates that the ACE/ACE2 ratio regulates the production and accumulation of AngII and that ACE2 deficiency leads to increased AngII concentrations (Ye et al. 2004; Wakahara et al. 2007).

There is emerging evidence of the upregulation of ACE2 in urine from diabetic patients; this upregulation may reflect the pathological shedding of renal ACE2. Studies in experimental models have investigated the feasibility of pharmacological induction of ACE2 for improvement of renal function, inflammation, and fibrosis (Williams and Scholey 2018). The small-molecule ACE2 activator 1-[[2-(dimethylamino)ethyl] amino]-4-(hydroxymethyl)-7-[[(4-methylphenyl)sulfonyl]oxy]-9H-xantona-9 (XNT) prevented renal and myocardial hydroxyproline accumulation (Paulis et al. 2015). Other ACE2 activators, such as diminazene aceturate (DIZE) and recombinant human ACE2, have been tested in preclinical trials (Treml et al. 2010; Ferreira et al. 2011).

33.4.2 MAS1 Agonists

Similar to ACE2 administration, Ang1-7 binding to the MAS1 receptor has shown beneficial effects in animal models. Further, Ang1-7 ameliorates cardiac remodeling by decreasing hypertrophy and fibrosis, while genetic depletion of Mas results in dyslipidemia, insulin resistance and marked fibrotic and hypertrophic changes in rat myocardia (Wiemer et al. 2002; Grobe et al. 2007).

The major limitation of exogenous administration of Ang1-7 is that it is a peptide with a very short biological half-life, low oral bioavailability, and very low stability (Yamada et al. 1998). Because of these limitations, Ang1-7 is generally administered subcutaneously by osmotic mini pumps, which are quite expensive and are not readily available.

More than 10 years ago, the effects of nonpeptide compound AVE 0991 were similar to those of Ang1-7 in endothelial cells (Wiemer et al. 2002). Subsequent data demonstrated that AVE 0991 and Ang1-7 competitively bound the same MAS1 receptor in the kidneys, and AVE 0991 reduced blood pressure, alone or in combination with renin inhibitors (Singh et al. 2013). In 2014, AVE 0991 was shown to ameliorate kidney inflammation and improve cell infiltration, cytokine release, and histology in two rodent models of arthritis. In diabetic animal models, AVE-0991 produced cardio-renal protection, possibly by improving glucose and lipid metabolism in diabetic rats, independent of its

blood pressure-lowering action (Singh et al. 2012). The first phase of clinical trials is being conducted to test the safety of combination therapy in humans (R. A. Santos, personal communication). Further clinical trials should be performed to test this promising therapeutic approach in humans (Barroso et al. 2012).

33.5 New Drugs of the AngIV-AT4R Axis

33.5.1 Aminopeptidase A

AngII is increased in the plasma and tissues either by increased ACE expression and activity or by decreased expression and activity of key enzymes that metabolize AngII. A balance between the formation and degradation of the effector peptide AngII is critical. The roles of AngII degradation in blood pressure and renal regulation have not been well studied. AngII is metabolized to form des aspartyl1-AngII, also called AngIII, primarily by aminopeptidase A (APA), which is involved in the degradation of AngII.

APA is expressed in glomerular podocytes and tubular epithelia and metabolizes AngII, a peptide known to promote glomerulosclerosis. APA activity is involved in the deterioration of salt-induced hypertension and renal injury (Nomura et al. 2005). Since APA is the key enzyme for the degradation of AngII, its implications for renal diseases have been studied in APA-knockout mice. AngII-treated APA-null mice developed a significant rise in albuminuria along with increased segmental and global sclerosis and/or collapse of juxtamedullary glomeruli, micro-cystic tubular dilation, and tubulo-interstitial fibrosis, which are blocked in AngII-treated APA-wild-type mice. The augmented AngII-mediated kidney injury observed in association with increased intrarenal AngII accumulation in the absence of APA suggests a protective, metabolizing role of APA in AngII-mediated glomerular diseases (Velez et al. 2014, 2017).

However, the clinical benefits of APA as a therapeutic target are most likely be limited, since it acts to decrease circulating and tissue AngII levels primarily by degrading AngII rather than by inhibiting AngII formation. ACE inhibitors have been widely used to block the RAAS to treat hypertensive and kidney diseases with proven clinically beneficial outcomes. If ACE inhibitors are inadequate to treat hypertension or kidney diseases associated with the formation of AngII, a potential pharmacological strategy to upregulate APA expression or enhance its activity may be alternative therapy (Gao et al. 2014).

QGC001 (originally named RB150) is the first drug candidate of a new class of antihypertensive agents targeting the brain RAAS, particularly brain APA, the enzyme generating brain AngIII. The phase I study of QGC001 showed that QGC001 offered a potential alternative therapeutic strategy for improving the BP control of hypertensive patients. However, the renoprotection of QGC001 warrants further investigation (Balavoine et al. 2014).

33.5.2 AT4 Receptor Inhibitors

The AT4 receptor (AT4R) was originally defined as the specific, high-affinity binding site for the hexapeptide angiotensin IV (AngIV). AT4R has a broad distribution and is found in a range of tissues, including the adrenal gland, kidney, lung, and heart. AT4R was generally found to be more abundant than AT1R in mammalian kidneys, whereas the Ang1-7 receptor was not detected in mammalian kidneys. Rats subjected to various chronic treatments were found to preferentially present decreased kidney AT4R density (furosemide, puromycin aminonucleoside, and nitro-L-arginine methylester), decreased kidney AT1R density (bilateral ureteral obstruction), or increased kidney AT1R distribution in the inner medulla (water diuresis). These results suggest that AT4R can be expressed in several renal cells within the normal kidney. Furthermore, many animal models of renal dysfunction and injury have been identified that selectively alter kidney AT4R density and may potentially aid in elucidating the role of this novel angiotensin receptor system in renal function (Handa et al. 2001).

IRAP, an AT4R that is inhibited by AngIV, was identified in 2001. A previous study demonstrated that IRAP is an AT4R and proposed that AT4R ligands may exert their effects by inhibiting the catalytic activity of IRAP, thereby extending the half-life of its neuropeptide substrates. HFI-419 is an IRAP-selective pyridinyl compound that enhances memory in rats. Studies have demonstrated that HFI-419 prevented cardiac and endothelial damage induced by AngII, independent of blood pressure. HFI-419 also exceeded the anti-inflammatory effect. Regarding the renoprotection of HFI-419, more studies are necessary (Albiston et al. 2008).

33.6 New Drugs Targeting Aldosterone

33.6.1 Nonsteroidal MRAs

Finerenone (BAY 94-8662) is a potent and highly selective nonsteroidal MRA (>500-fold more selective than for other steroid receptors) (Taira et al. 2008). Lattenist et al. (2017) evaluated the efficacy of finerenone to prevent the acute and chronic consequences of ischemic acute kidney injury. After 4 months of ischemia-reperfusion, finerenone fully prevented the transition from acute kidney injury to chronic kidney disease (kidney dysfunction, increased proteinuria and tubular dilation, extensive tubule-interstitial fibrosis, and an increase in kidney TGF-β and collagen I mRNA). Moreover, Barrera-Chimal J's lab (Barrera-Chimal et al. 2018) also reported that finerenone efficiently prevented IR-induced increases in plasma creatinine, urea, and proteinuria levels, as well as the expression of TGF-β as an indicator of kidney fibrosis, which means that finerenone protected against the transition from acute kidney injury to chronic kidney disease. Furthermore, they found that MR deficiency in myeloid cells protected against chronic dysfunction and fibrosis induced by ischemia-reperfusion. Finally, they found that the protection afforded by MR

antagonism (finerenone) or myeloid MR deficiency was due to the promotion of macrophage polarization to a wound-healing phenotype after kidney IR, preventing the development of chronic kidney fibrosis and dysfunction.

Arai K and his colleagues (Arai et al. 2016) observed, in a hypertensive rat model, that esaxerenone (CS-3150), a novel nonsteroidal mineralocorticoid receptor antagonist, not only prevented but also ameliorated ongoing DOCA/salt loading-induced mRNA expression of fibrosis, inflammation, and oxidative stress markers. Moreover, they found that compared with spironolactone and eplerenone, CS-3150 had an equivalent antihypertensive effect but a superior ability to ameliorate glomerulosclerosis, tubular injury, and tubulo-interstitial fibrosis (Arai et al. 2015).

33.6.2   *Aldosterone Synthase Inhibitor*

Aldosterone is synthesized from cholesterol in the outermost layer of the adrenal cortex via a series of steroid hydroxylase and deoxygenase enzymes. Aldosterone synthase (also named CYP11B2) catalyzes the last and rate-limiting steps in aldosterone synthesis. The major glucocorticoid, cortisol, is synthesized in the zona fasciculata of the adrenal cortex, with CYP11B1 (11β-hydroxylase (cytochrome P450 type I)) as the rate-limiting enzyme. It is well known that aldosterone and cortisol biosynthesis share many common steps (Azizi et al. 2013; Tamargo et al. 2014).

Aldosterone stimulates the production of ROS, inflammation and fibrosis of the heart, vasculature, and kidney through both mineralocorticoid receptor (MR)-dependent and MR-independent mechanisms. Among them, the MR-independent effects occur via the angiotensin II receptor and via the G-protein-coupled receptor. Studies in rodents genetically deficient in aldosterone synthase or treated with a pharmacological aldosterone synthase inhibitor have provided insight into the relative contribution of aldosterone compared with the contribution of mineralocorticoid receptor activation in inflammation, fibrosis, and injury (Brown 2013).

LCI699 is an orally active and nonselective aldosterone synthase inhibitor that has been evaluated in humans (Andersen et al. 2012). With aldosterone synthase inhibition by LCI699, cortisol levels remain normal, and 11-deoxycorticosterone increases, which means that the adrenocorticotropic hormone–cortisol axis is activated by CYP11B1 gene inhibition. However, the effects of aldosterone synthase inhibition on renal injury have not been reported in humans to date (Brown 2013).

33.7   Combination Strategy

Despite the broad range of new possible therapeutic targets for hypertension described above, it is seemingly difficult to devise a new molecule that can be advanced to later phases of clinical investigation and that could successfully compete with the existing therapeutics. Thus, there is much room to take advantage of

the already broad choice of molecules and to optimize their usage. The combination of current drugs is an alternative strategy. The use of combination therapy for the treatment of renal fibrosis is already established in practice and in the current guidelines. This positive effect has been amply confirmed for ACEI or ARB used as monotherapy, and two or more separate RAAS drugs have been combined in the hope that they would have an antifibrotic effect and offset each other's negative side effects.

33.7.1 ACEI + ARB Combination

In theory, the combination of RAAS inhibitors may have a greater inhibitory effect of renal fibrosis and a better clinical outcome. However, the combination use of ACEI and ARB may have synergistic or addictive effects. A meta-analysis found that a combination of ACEI and ARB resulted in a clinically significant reduction in proteinuria in patients with chronic kidney disease and diabetic nephropathy regardless of BP changes (Doulton and Macgregor 2005). Another systematic review and meta-analysis of randomized trials evaluating the combination of an ACEI and an ARB in patients with chronic proteinuric renal disease. This finding indicated that the combination of ACEI and ARB therapy in patients with chronic proteinuric renal disease was safe, without clinically meaningful changes in serum potassium levels or glomerular filtration rates. Combination therapy was also associated with a significant decrease in proteinuria, at least in the short term (MacKinnon et al. 2006). However, Schmerbach et al. (2012) observed the effect of ACEI/ARB combination therapy in spontaneously hypertensive stroke-prone rats on a salt-rich diet. Renal glomerulosclerosis and interstitial fibrosis were decreased by monotherapy (telmisartan or ramipril), whereas combination treatment (telmisartan and ramipril) failed to have a significant effect. In the ONTARGET trial, the combination therapy of ramipril plus telmisartan was compared to monotherapy in patients with DM and high cardiovascular risk. There was no difference in the composite primary outcome of death or hospitalization for cardiovascular disease, but combination therapy was associated with significantly more hypotension, syncopes, and a faster rate of decline in renal function compared to monotherapy. The ONTARGET trial also showed that dual RAAS blockade led to worsening of kidney function (Mann et al. 2008). As with ONTARGET, the VA NEPHRON D Trial, targeting patients with T2DM, compared combination therapy of losartan and lisinopril to monotherapy with losartan. Due to excess adverse events in the combination arm of ACEI and ARB, mostly acute kidney injury and hyperkalemia, the trial was prematurely stopped (Fried et al. 2013).

33.7.2 ACEI/ARB + MRA Combination

MRAs are used in combination with ACEI or ARB, particularly in patients with low renin or resistant hypertension, which is effective in reducing BP because of the inability of these drugs to reliably decrease aldosterone after chronic use as a consequence of the aldosterone breakthrough that follows the escape of the effects of ACEI or ARB. Eplerenone is also effective in resistant hypertension in association with ACEI or ARB. The demonstrated better tolerability of eplerenone over spironolactone could make the use of eplerenone more desirable, but the indication for hypertension is not recognized except in the presence of intolerance to spironolactone (Marquez et al. 2015).

Mehdi et al. (2009) published a double-blind, placebo-controlled trial that included 81 patients with diabetes, hypertension, and albuminuria (UAE > 300 mg/g). Patients who were treated with lisinopril 80 mg were randomized to placebo, losartan 100 mg or spironolactone 25 mg daily for 48 weeks. The results revealed that compared with placebo, the urinary albumin-to-creatinine ratio decreased by 34.0% in the spironolactone group and by 16.8% in the losartan group. However, the serum potassium level was significantly higher with the addition of either spironolactone or losartan, which indicated that the addition of spironolactone, but not losartan, to a regimen including a maximal dose of an ACEI generated better renoprotection in patients with diabetic nephropathy. Epstein et al. published similar RCT results in 2006 (Epstein et al. 2006). Additional large-scale trials with hard end points are needed in this field to prove that the addition of an aldosterone receptor blocker to standard therapy with an ACEI or ARB could improve renal outcomes, and the effect of the new MRA on renoprotection should be analyzed.

33.7.3 Dual Blockade of ARB-Neprilysin Inhibitor

Natriuretic peptides (NP) (especially ANP and BNP), playing an important role in sodium and water homeostasis, have antioxidant, anti-inflammatory, and antifibrotic properties, which may significantly contribute to their renoprotective properties. Neprilysin is a vasopeptidase that metabolizes NP and other peptides, such as bradykinins. Inhibition of neprilysin is accompanied by the increase in NP levels but has mild effects on BP (MacKinnon et al. 2006). The combination of a neprilysin inhibitor with a RAAS blocker represents a powerful tool. LCZ696 is a dual-acting ARB and neprilysin inhibitor (ARNI) that decreases BP in animals and healthy humans with low rates of side effects. Therefore, it is not surprising that in CKD animals treated with LCZ, there was a significant improvement in indices of oxidative stress, inflammation, fibrosis, and the Nrf2 system beyond that observed with ARB therapy alone. In a clinical study, a meta-analysis of LCZ696 revealed that it was better than ARB alone at reducing most blood pressure parameters without resulting in more adverse events in treating hypertension (Jing et al. 2017). To determine the

renoprotective effect of LCZ696, additional clinical trials should address its ability to prevent renal fibrosis and the safety of its long-term use.

33.7.4   ACEI/ARB + DRI Combination

With respect to preclinical studies, the result of the ALTITUDE study has been unexpected; in particular, a renoprotective effect was expected from the aliskiren + ACEI/ARB combination. This combination therapy might reduce proteinuria to a great extent. However, further subgroup analysis revealed that dual blockade of the renin–angiotensin–aldosterone system with aliskiren and ARB did not improve the hard renal endpoints, so it should not be recommended in CKD patients (Parving et al. 2012; Rasche et al. 2018).

33.8   Conclusions

For years, a large number of experimental and clinical trials have shown that the traditional RAAS blocker (ACEI/ARB/MAR) could delay the development of renal fibrosis and chronic kidney disease, but the effect was limited. Improvements in RAAS blockade beyond the effects of ACEIs and ARBs will be challenging, but studies in this area are abundant. Some new nonpeptide drugs that modify RAAS activity to combat renal fibrosis have been developed, including several novel agonists (such as AT2R agonists and MAS1 agonists) and antagonists (such as AT4R inhibitors) of the RAAS, some of which have entered clinical applications and some of which are still in the stage of clinical trials. Furthermore, with the development of new technology, new blockers of the RAAS have been developed, such as human recombinant ACE2 and vaccines against renin, AngI or AngII, which may be more specific. However, large multicenter randomized controlled studies are needed to evaluate the beneficial effects and to explore the efficacy and safety of these new blockers of the RAAS.

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