Minireview

New agents modulating the renin-angiotensin-aldosterone system—Will there be a new therapeutic option?

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Abstract

The renin-angiotensin-aldosterone system (RAAS) is more complex than it was originally regarded. According to the current subject knowledge, there are two main axes of the RAAS: (1) angiotensin-converting enzyme (ACE)-angiotensin II-AT_1 receptor axis and (2) ACE2-angiotensin-(1-7)-Mas receptor axis. The activation of the first axis leads to deleterious effects, including vasoconstriction, endothelial dysfunction, thrombosis, inflammation, and fibrosis; therefore, blocking the components of this axis is a highly rational and commonly used therapeutic procedure. The ACE2-Ang-(1-7)-Mas receptor axis has a different role, since it often opposes the effects induced by the classical ACE-Ang II-AT_1 axis. Once the positive effects of the ACE2-Ang-(1-7)-Mas axis were discovered, the alternative ways of pharmacotherapy activating this axis of RAAS appeared. This article briefly describes new molecules affecting the RAAS, namely: recombinant human ACE2, ACE2 activators, angiotensin-(1-7) peptide and non-peptide analogs, aldosterone synthase inhibitors, and the third and fourth generation of mineralocorticoid receptor antagonists. The results of the experimental and clinical studies are encouraging, which leads us to believe that these new molecules can support the treatment of cardiovascular diseases as well as cardiometabolic disorders.

Keywords: Angiotensin converting enzyme 2, angiotensin-(1-7), Mas receptor, finerenone, mineralocorticoid receptor coregulators, aldosterone synthase inhibitors

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Introduction

The renin-angiotensin-aldosterone system (RAAS) regulates blood pressure and fluid and electrolyte balance under physiological conditions. However, it plays also a role in pathological processes leading to cardiovascular and other disorders, e.g. hypertension, coronary artery disease, heart failure, and kidney diseases. The drugs blocking the RAAS, e.g. angiotensin-converting enzyme inhibitors (ACE-Is), angiotensin II receptor blockers (ARBs) and mineralocorticoid receptor antagonists (MRAs), are the mainstays of current pharmacotherapy for cardiovascular diseases (CVD).[1,2] Moreover, some data suggest the pleiotropic effects of RAAS blockers, since beneficial clinical effects of RAAS inhibition in atherosclerosis, atrial fibrillation, post-ischemic stroke state, pulmonary hypertension, diabetic vasculopathy, Alzheimer's disease, or tumor angiogenesis were observed.[3–5] Thus, it seems that in the near future, pharmacotherapy with RAAS-affecting agents could be extended to new indications. On the other hand, standard therapeutic procedure in CVD based on ACE-Is, ARBs, and MRAs appears to be insufficient sometimes, since it blocks only certain elements of the RAAS, while other pathways are still unaffected. This may lead to a phenomenon called 'RAAS escape', which may attenuate the clinical benefit of RAAS blockade. 'RAAS escape' was observed during either ACE-Is or ARBs treatment, when renin and angiotensin I (Ang I) accumulation overcame the ability of drugs to effectively suppress RAAS.[6] Moreover, there are also ACE-independent pathways of Ang II formation. The reactivated Ang II promotes aldosterone secretion. 'Aldosterone escape' occurs during long-term ARBs therapy, as well as due to increased serum potassium levels or angiotensin II receptor type 2 (AT_2)-dependent mechanism.[7] Furthermore, the broad use of drugs blocking the RAAS has been limited by significant incidence of side effects, e.g. hyperkalemia, which forces research for new drugs expressing adequate efficacy while avoiding the adverse effects.[8] Taking all these factors into account, no wonder scientists

worldwide try to understand the functions of the RAAS better and to discover new drugs modulating it, as well as expand their indications. Indeed, the new molecules affecting the RAAS, namely: recombinant human ACE2 (rhACE2), ACE2 activators, angiotensin-(1-7) peptide and non-peptide analogs, aldosterone synthase inhibitors (ASIs) and the third and fourth generation of MRAs, have been described recently. Some of these new compounds are in clinical trials already. The question arises, whether the new agents modulating the RAAS will be new drugs in the future. The results of the experimental and clinical studies are encouraging, which leads us to believe that these new molecules can support the treatment of CVD.

Two axes of RAAS and their functions

According to the current subject knowledge, there are two main axes of the RAAS: (1) ACE-angiotensin II-AT_1 receptor axis and (2) ACE2-angiotensin-(1-7)-Mas receptor axis (Figure 1).[9,10] Activation of the ACE-Ang II-AT_1 causes deleterious effects, including vasoconstriction, endothelial dysfunction, thrombosis, inflammation, and fibrosis.[11] ACE2-Ang-(1-7)-Mas receptor axis has a different role, since it often opposes the effects induced by the classical ACE-Ang II-AT_1 axis.[12] It has been suggested that the activity of the RAAS and the actions of RAAS blockers depend on the balance between these two axes. It was demonstrated that therapy with ACE-Is and ARBs prevented the decrease in ACE2 expression in myocardial infarcted rats and increased plasma Ang-(1-7) levels.[13–15] This could be evidence that these drugs are effective not only due to the inhibition of Ang II effects, but also due to the activation of the ACE2-Ang-(1-7)-Mas axis of the RAAS. Since, the positive effects of the ACE2-Ang-(1-7)-Mas axis were discovered, the alternative ways of pharmacotherapy activating this axis of RAAS appeared. ACE2-Ang-(1-7)-Mas signaling, called 'vasoprotective axis' as well, has the potential to be considered a novel therapeutic approach to counterbalance the ACE-Ang II-AT_1 axis as a novel approach targeting RAAS.[10] Indeed, there are researched exogenous Ang-(1-7) analogs and ACE2 activators, which may be effective in the treatment of CVD, prevention and treatment of diabetic vasculopathy or metabolic syndrome.

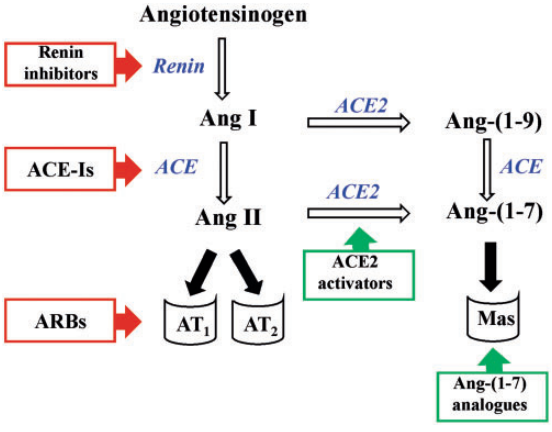

Figure 1 Targets of molecules affecting the RAAS. In red frames—drugs blocking the ACE-AngII-AT_1 axis, in green—molecules activating the ACE2-Ang-(1-7)-Mas axis. Ang: angiotensin, ACE: angiotensin I converting enzyme, ACE-Is: angiotensin I converting enzyme inhibitors, ACE2: angiotensin I converting enzyme type 2, ARBs: angiotensin II receptor type 1 blockers, AT_1: angiotensin II receptor type 1, AT_2: angiotensin II receptor type 2, Mas: Mas receptor. (A color version of this figure is available in the online journal.)

Angiotensin-(1-7)

Angiotensin-(1-7) is the element of the RAAS arousing big interest due to its opposite to Ang II action.[16,17] The presence of Ang-(1-7) has been confirmed in the heart, blood vessels, kidneys, and liver.[18] Ang-(1-7) is formed mainly through the removal of the C-terminal phenylalanine from Ang II by the action of ACE2. Ang-(1-7) can be also produced from Ang-(1-9) by ACE and neutral-endopeptidase. Ang-(1-7) exerts its action through stimulation of the specific G-protein coupled Mas receptor. Receptor Mas stimulation leads, among others, to increased phosphorylation of endothelial nitric oxide synthase (eNOS) and increased nitric oxide (NO) release. In addition, it augments prostacycline synthesis and suppresses the release of norepinephrine and thus Ang-(1-7) is considered a vasodilating and antiarrhythmogenic factor.[12,19] The effects of Ang-(1-7) in diabetes and its cardiovascular disorders are a new research area, although there are already some data confirming the positive impact of this peptide on glucose metabolism and its role in the prevention of the hyperglycemia-induced disorders. It was demonstrated that Mas-knockout mice presented changes in glucose and lipid metabolism which ended up with a condition resembling metabolic syndrome, while chronic elevation of Ang-(1-7) levels in transgenic rats leads to better glucose tolerance and insulin sensitivity, a decrease in the plasma triglyceride and cholesterol levels and a reduction in adipose tissue mass.[20,21] The possible mechanism of Ang-(1-7) action in glucose metabolism may be related to the modulation of blood flow and inhibition of fibrosis and therefore glucagon and insulin release.[22] The protective role of Ang-(1-7) in the cardiovascular disorders of diabetes was also observed. It was demonstrated that Ang-(1-7) attenuates diabetic cardiomyopathy in rats because of vasodilatory, antiproliferative, and antifibrotic properties.[23–26] Furthermore, the cardioprotective effect of this peptide was also related to a decrease in dyslipidemia. However, the therapeutic use of Ang-(1-7) is limited due to its unfavorable pharmacokinetic properties.[27] Thus, new strategies (e.g. the use of cyclodextrins, liposomal delivery systems, modifications of the peptide—cyclic form) are sought to make clinical application of Ang-(1-7) possible.[28]

Non-peptide Ang-(1-7) analogs

The most widely studied so far Ang-(1-7) analog is AVE 0991, which is a non-peptide, orally active and physiologically well tolerated imidazole derivative.[29] Despite the fact that the first studies on AVE 0991 come from the last decade, there are only few publications demonstrating the pharmacodynamics and pharmacokinetics of this agent.

In 2002, the first *in vitro* study of AVE 0991 took place.[30] It was demonstrated that novel compound caused a subsequent increase in NO and low concomitant production of

Table 1 New agents modulating RAAS in the experimental studies

New agents in RAAS	Beneficial effects observed in animal models	Target diseases—potential clinical implication	Refs.
Non-peptide Ang-(1-7) analogs (AVE 0991)	• Decrease in infarcted area in rats with myocardial infarction • Inhibition of atherogenesis in apoE-knockout mice • Reduction of endothelial dysfunction in salt-fed rats • Anti-hypertensive effect in 2K1C and DOCA hypertensive rats • Improvement in hemodynamics and renal protection in streptozotocin-induced diabetic rats	• Myocardial ischemia • Heart failure • Atherosclerosis • Hypertension • Diabetes	31–40
Peptide Ang-(1-7) analogs (CGEN-856S)	• Decrease in blood pressure in SHR rats • Decrease in infarcted area and cardiac remodelling in rats with hypertrophy and myocardial infarction	• Hypertension • Myocardial ischemia • Arrhythmias	41–42
rhACE2	• Reduced inflammation, renal dysfunction, and glomerulus injury in apoE-knockout mice • Reduced hypertrophy, diastolic dysfunction, and myocardial fibrosis in mice with hypertrophy and diastolic dysfunction	• Atherosclerotic renal injury • Kidney diseases • Heart failure	60–62
ACE2 activators (Xanthenon, DIZE)	• Decrease in blood pressure in SHR rats • Reduction in interstitial fibrosis in rats with pulmonary hypertension • Reduction in thrombus weight and area in rat venous thrombosis • Improvement in the autonomic and cardiac dysfunction in streptozotocin-induced diabetic rats	• Hypertension • Diabetes with cardiovascular autonomic dysfunction	64–68
MRAs (Finerenone)	• Improved mortality and nephroprotection in SHR stroke-prone rats • Cardiorenal protection in rats with diastolic heart failure	• Hypertension • Heart failure	102–104
ASIs (FAD286)	• Reduced cardiac hypertrophy, oxidative stress and improved hemodynamics and endothelial function in rats with post-myocardial infarction heart failure • Reduced lesion area and inflammation in apoE-knockout mice • Reduced renal inflammation, albuminuria in streptozotocin-induced diabetic rats • Lowered blood pressure in hypertensive salt-fed rats • Reduced neovascularization and retinopathy in rat model of retinopathy	• Congestive heart failure • Atherosclerosis • Diabetic nephropathy • Hypertension • Retinal neovascularisation	110–121

O_2 in bovine aortic endothelial cells. AVE 0991 caused approximately five times higher release of bioactive NO compared with Ang-(1-7). Moreover, it was demonstrated that the effects of AVE 0991 were not completely abolished by inhibition of NOS or blockade of AT_1 and AT_2 receptors.[30] The beneficial effects of AVE 0991 were confirmed in various experimental models of CVD and diabetes (Table 1).[31–40] Despite the promising results of experimental studies, the development of AVE 0991 has been stopped for unknown strategic reasons.

Peptide Ang-(1-7) analogs

Peptide Ang-(1-7) analogs, natural ligands able to stimulate G-protein coupled receptors (Mas among others), were discovered during the human proteome analysis. As a result, two peptides, CGEN-856 and CGEN-857, were examined (amino acid sequence FLGYCIYLNRKRRGDPAFKRRLRD and SMCHRWSRAVLFPAAHRP, respectively). What is the most important is that the compounds have no significant homology to Ang-(1-7) or to known G-protein coupled receptor ligands. These peptides have several chemical structures, but CGEN-856S (a monomer in which cysteine was substituted with serine) displays the highest affinity for the Mas receptor confirmed in experimental *in vitro* and *in vivo* models. CGEN-856S displays high, like AVE 0991, affinity for the Mas receptor.[41] The favorable effects of CGEN-856S in the cardiovascular system were confirmed in animal models of CVD (Table 1).[41,42]

Ang-(1-7) analogs in clinical trials

A major limitation of Ang-(1-7) use is that this molecule is a peptide with a short plasma half-life and is rapidly degraded in the gastrointestinal tract when given orally. Although, some attempts to make Mas stimulation suitable for clinical use of orally active derivatives of Ang-(1-7) are being made. Some of the Ang-(1-7) analogs entered the clinical studies, including NorLeu^3-Ang-(1-7) which is currently studied as DSC127 for topic treatment of diabetic

foot ulcers (DFU) (Table 2).[43,44] DFU patients are being recruited into phase III clinical trials for DSC127 (NCT01830348 and NCT01849965).[45] One pharmaceutical company aims to initiate clinical trials with another Ang-(1-7) analog—TXA127 in patients with Duchenne muscular dystrophy or congenital muscular dystrophy in early 2016. So far, the positive effects of TXA127 in muscle dystrophy, including reduction in muscle fibrosis, increases in muscle strength as well as normalization of cardiac dysfunction, were confirmed in experimental models.[46–48]

Table 2 New agents modulating RAAS in the clinical studies

	Clinical study				
New agents in RAAS	**Phase/Acronim**	**Patients**	**Results**	**Mechanism of action**	**Ref.**
Ang-(1-7) analogs (DSC127)	Phase II clinical study	Patients with chronic, noninfected, neuropathic, or neuroischemic plantar Wagner Grade 1 or 2 foot ulcers	• Safety and efficacy of DSC127 in accelerating the healing of diabetic foot ulcers	• Induction of progenitor proliferation • Accelerated vascularisation	43
rhACE2	Phase I clinical study	Healthy volunteers	• Determined pharmacokinetics, pharmacodynamics, safety, and tolerability • Lack of cardiovascular effects despite of marked changes in angiotensin system peptide concentrations	• The presence of effective compensatory mechanisms in healthy volunteers is suggested	63
MRAs (Finerenone)	Phase IIa clinical study (ARTS)	Patients with heart failure associated with a reduced left ventricular ejection fraction and chronic kidney disease	• Determined pharmacokinetics, pharmacodynamics, safety, tolerability, and optimal dose range for further studies • Less hyperkalemia and renal dysfunction compared with spironolactone	• Greater selectivity than spironolactone and stronger mineralocorticoid receptor binding affinity than eplerenone	105
	Phase IIb clinical study (ARTS-DN)	Patients with type 2 diabetes and clinical diagnosis of diabetic nephropathy	• Well tolerated with good safety profile • Future long-term clinical studies examining the effects of finerenone on the progression of renal disease as well as on CV morbidity and mortality in patients with DKD are needed		106
	Phase IIb clinical study (ARTS-HF)	Patients with worsening chronic heart failure and reduced left ventricular ejection fraction and at high risk of hyperkalemia and worsening renal dysfunction	• Investigated the safety and potential efficacy finerenone in a high-risk population of patients • Assessed the effects of finerenone on a composite clinical endpoint		107
ASIs (LCI699)	Phase II clinical study	Patients with primary aldosteronism	• Decreased plasma aldosterone concentration • Corrected the hypokalemia and mildly decreased blood pressure	• Effectively and safely inhibited aldosterone synthase (CYP11B2)	123
	Phase II clinical study	Patients with primary hypertension	• Lowered clinic and ambulatory blood pressure	• Effectively and safely inhibited aldosterone synthase (CYP11B2)	124
	A multicenter, proof-of-concept study	Patients with Cushing's disease	• Efficacious and well tolerated in patients with Cushing's disease • Normalized urinary cortisol • Decreased blood pressure	• Inhibited cortisol synthesis (CYP11B1)	125

Angiotensin converting enzyme 2

A monocarboxypeptidase, angiotensin-converting enzyme 2 (ACE2) is 42% homolog to ACE1 and is expressed in the heart, kidney, testis, endothelium of coronary, intrarenal vessels, and renal tubular epithelium.[12] ACE2 shows a 400-fold higher affinity to Ang II than to Ang I. ACE2 produces vasodilator peptides Ang-(1-7) from Ang II (Figure 1). ACE-Is increase angiotensin-(1-9) and Ang-(1-7) levels, which is probably related with the enhanced activation of ACE2.[12] Moreover, ACE2 is a target for severe acute respiratory syndrome coronavirus (SARS)-CoV. During infection with this virus the expression of ACE2 is decreased, which probably contributes significantly to the development of pulmonary insufficiency.[49] ACE2 is activated in the heart ventricles of primary pulmonary hypertension patients, which suggests that ACE2 could be a cardioprotective enzyme.[50] Indeed, the results of experimental studies support this thesis. Studies in rats overexpressing ACE2 showed a reduction in blood pressure and an improvement in endothelial function.[51] It has been also demonstrated that lack of the ACE2 gene leads to an increase in adhesion molecules and proinflammatory cytokine expression, which augments vascular inflammation and atherogenesis in ApoE knockout mice.[52] Moreover, the benefits of ACE2 in experimental models of diabetes were demonstrated. Infection with the adenovirus containing human ACE2 gene resulted in improved fasting glycemia and glucose tolerance, an increase in pancreatic β cells proliferation and limitation of their apoptosis in diabetic mice.[53] Moreover, it was demonstrated that ACE2 plays a protective role in diabetic nephropathy in experimental animals.[54,55] According to the latest reports, changes in ACE2 gene expression were observed during clinical studies in type 1 and type 2 diabetic patients.[56,57] There was a positive correlation between the ACE/ACE2 ratio and such variables as blood pressure, fasting glycemia, creatinine levels, and urine protein.

The presented data proves a potential ACE2 role in the prevention of CVD and organ damage provoked by sustained hyperglycemia, thus the search for new molecules and methods of modulating ACE2 activation is required.

Recombinant human ACE2

One of the possibilities activating the ACE2-Ang-(1-7)-Mas axis is the use of rhACE2. It was demonstrated that treatment of Ang II-infused wild-type mice with rhACE2 blunted the hypertrophic response and expression of hypertrophy markers and reduced Ang II-induced superoxide production and Ang II-mediated myocardial fibrosis.[58] These effects were associated with reduced plasma and myocardial Ang II and increased plasma Ang-(1-7) levels. Importantly, rhACE2 partially prevented the development of dilated cardiomyopathy in pressure-overloaded wild-type mice.[58] These data prove that ACE2 is an important negative regulator of Ang II-induced heart disease and can suppress adverse myocardial remodeling. The beneficial effects of rhACE2 were also demonstrated in experimental models of diabetic kidney injury in association with a reduction in blood pressure and a decrease in oxidative stress.[59] Moreover, blocking Ang-(1-7) action prevents the beneficial effects of rhACE2 leading to systolic dysfunction.[60] These results highlight a key cardioprotective role of Ang-(1-7) and potential therapeutic strategy for CVD (Table 1).[61,62] Actually, rhACE2 was successfully taken through a phase I trial and was well tolerated by healthy volunteers. Although, despite marked changes in angiotensin peptide concentrations, cardiovascular effects were lacking, suggesting the presence of some compensatory mechanisms in healthy volunteers (Table 2).[63]

ACE2 activators

The second way to increase ACE2 activity, and therefore Ang-(1-7) synthesis, is to use agents modulating ACE2 gene expression. In 2008, two ACE2 activators were discovered: xanthenon (XNT) and resorcinolnaphthalein. *In vitro* studies showed that these two compounds in a dose-dependent manner enhanced ACE2 activity by approximately two-fold from control levels.[64] However, due to the results of a solubility study only XNT was researched *in vivo.* XNT is significantly more soluble than resorcinolnaphthalein, thus it was commonly used in *in vivo* studies. The protective cardiovascular effects of XNT were confirmed in various animal models of CVD and diabetes (Table 1).[64–68]

Recently, an antitrypanosomal drug, diminazene aceturate (DIZE), was shown to exert an ''off-target'' effect of enhancing the activity of ACE2 *in vivo*. The potential benefits of DIZE in the therapy of hypertension and its complications were demonstrated in different animal models (Table 1).[69–73] The protective effects of DIZE were associated with the activation of the vasoprotective axis of the lung RAAS, decreased inflammatory cytokines, improved pulmonary vasoreactivity, and enhanced cardiac function.[69] A recent report demonstrated that the mechanism of DIZE's antihypertensive action involves Mas receptor activation and the NO-dependent pathway.[70] Moreover, it was shown that treatment with DIZE improved hypercholesterolemia-induced corpus cavernosum injury, suggesting ACE2 as a potential target for treating erectile dysfunction.[72]

The cardioprotective properties of ACE2 activators could mean future use of these compounds in the prevention of cardiac insufficiency or diabetes complications, including hemostasis disturbances. These results, with the reduction of lipogenesis markers, open a new perspective for metabolic disorder pharmacotherapy. At the moment, the effects of ACE2 activators were evaluated only in preclinical studies.

Aldosterone

Aldosterone, the final product of the RAAS, plays a crucial role in the pathophysiology of the cardiovascular system.[74] Aldosterone contributes to endothelial dysfunction, fibrinolytic disorders, inflammation, oxidative stress, fibrosis, hypertrophy, and arrhythmias leading to progression of CVD.[75–78]

The blockade of aldosterone action has been demonstrated to be an extremely beneficial therapy in CVD.

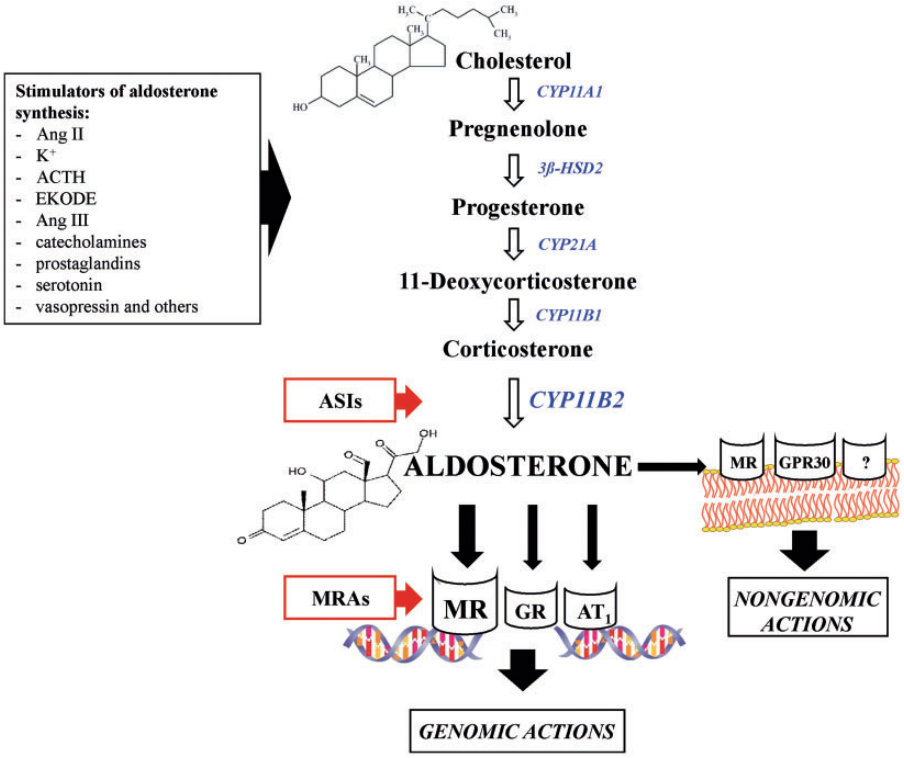

Figure 2 Aldosterone synthesis and targets of hormone blockers. ACTH: adrenocorticotropic hormone, Ang II: angiotensin II, Ang III: angiotensin III, ASIs: aldosterone synthase inhibitors, AT_1: angiotensin II receptor type 1, CYP11B2: aldosterone synthase, EKODE: oxidized derivative of linoleic acid, GPR30: estrogen receptor, GR: glucocorticoid receptor, K^+: potassium ions, MR: mineralocorticoid receptor, MRAs: mineralocorticoid receptor antagonists, '?': non-identified cell-membrane receptor. (A color version of this figure is available in the online journal.)

Clinical trials with spironolactone and eplerenone, steroidal MRAs, investigated the potential role of aldosterone and MRAs in a variety of CVD. These trials are a result of clinical interest in the significant function of aldosterone in the cardiovascular system, which became evident after publication of the outcomes of two clinical trials: Randomized Aldactone Evaluation Study (RALES) and Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS).[79,80] Moreover, numerous animal studies have shown that MR blockade reduces cardiovascular, renovascular, and cardiometabolic disorders associated with obesity and diabetes.[81–83] Moreover, the prothrombotic effect of aldosterone was showed in experimental models of thrombosis.[84–87] It was demonstrated that the hormone enhances venous thrombosis in normotensive rats in the mechanism involving primary hemostasis, fibrinolysis, NO, and oxidative stress-dependent pathways.[88] Furthermore, the MR blockade was not sufficient to reverse aldosterone effects in hemostasis. The other receptors, e.g. glucocorticoid receptor (GR) and AT_1, were also involved in the prothrombotic action of aldosterone.[89,90] These results show that the aldosterone action is more complex and involves not only MR activation as it was previously thought (Figure 2).

However, the molecular mechanism of aldosterone action is not completely understood. The effects of aldosterone are mediated via classic nuclear receptors (genomic actions of aldosterone) and cell-membrane receptors (non-genomic actions of aldosterone) with alternative pathways (activation of protein kinases or secondary messenger signaling cascades).[91,92] It was well documented that aldosterone in supraphysiological concentrations can also act via GR.[93] Recently, it has been demonstrated that another important receptor aldosterone acts on is G protein coupled estrogen receptor (GPR30). GPR30 activation plays an important role in aldosterone-mediated regulation of endothelial cell growth and in aldosterone's endothelial-mediated regulation of vasoreactivity.[94]

This multiple mechanism of aldosterone action points to the need of a search for new strategies of aldosterone blockade.

New aldosterone blockers

To date, only two steroidal MRAs have been clinically used. Spironolactone represents first generation of nonselective MRAs, while eplerenone corresponds to the second generation with significantly improved selectivity for MR over other steroid receptors.[95] The IC_{50} of eplerenone for MR (990 nmol/L) is over 10-fold less than for androgen, progesterone, and estrogen receptors.[96]]. Despite the irrefutable beneficial effects of spironolactone and eplerenone confirmed in patients with heart failure and kidney disease, the use of MRAs is limited by the risk of hyperkalemia, especially in patients with renal disorders.[79,80,97] In fact, hyperkalemia was reported in up to 36% among elderly heart failure outpatients. Hence, the risk of hyperkalemia was the strongest stimulus for further research with third

generation of MRAs, which are nonsteroidal, more cardioselective thus exerting less renal side effects.[98] Few pharmaceutical companies have nonsteroidal MRAs in clinical development. However, no clinical data have been published so far for MT-3995, SC-3150, LY2623091, and PF-03882845. Although, there are some data available from a phase II trial for finerenone (developmental code name BAY 94-8862), showing safety and efficacy in patients with heart failure and chronic kidney diseases.[99]

Finerenone—third generation of MRAs

Finerenone is a dihydropyridine derivative without L-type Ca^{2+} channel activity and with less relative affinity to other steroid receptors than currently available MRAs.[99] Finerenone has unique pharmacodynamics as a consequence of different molecular properties. Similar to spironolactone and eplerenone, finerenone competitively antagonizes the MR, although it shows more natriuretic effects since it exerts a 3–10-fold higher potency and efficacy with IC_{50} of 18 nmol/L with exceptional selectivity versus the GR, androgen and progesterone receptors (>500-fold).[99–101] Finerenone shows cardiac and renal protection, which was confirmed in preclinical studies in rats (Table 1).[102–104] Furthermore, the end-organ protective activity were more pronounced in finerenone-treated rats compared to eplerenone-treated animals.[102] These positive outcomes from preclinical studies were further confirmed in trials (Table 2). The safety and tolerability of finerenone was studied during the Mineralocorticoid-Receptor Antagonist Tolerability Study (ARTS) in patients with heart failure and mild/moderate chronic kidney disease.[105] Treatment with finerenone resulted in less hyperkalemia and slower renal dysfunction compared with spironolactone, whereas the other cardiac and renal parameters were at least similar. Further clinical studies with finerenone in patients with worsening chronic systolic heart failure and type 2 diabetes and/or chronic kidney disease (ARTS-HF, ARTS-DN) showed positive outcomes as well.[106,107] However, the long-term effects of finerenone will be investigated in a phase III study for the treatment of chronic heart failure.

Fourth generation of MRAs

While MR blocking in the cardiovascular tissues is particularly sought after, the fourth generation of MRAs, presenting high tissue selectivity (cardiovascular over renal effects) and a renal-sparing profile (combined Na^+ excretion with a mild K^+ retention), is now postulated. This tissue selectivity can be achieved by improving the physiochemical properties of MRAs that alter their tissue distribution or by the interaction of novel MRAs with ''coregulator molecules''. There is evidence that coregulators, a heterogeneous group of nonreceptor proteins, are required to influence nuclear receptor-mediated transactivation of target genes.[108] It is expected that the interaction of novel MRAs with certain coregulators may allow the modulation of MR activity and selectivity. Thus, rather selective MR modulators, than MR blockers *per se*, may be a key factor in proper MR antagonism. Understanding the nature of MR-coregulator interactions may be a stimulator for a rational design of the fourth generation of MRAs.

Aldosterone synthase inhibitors

Bearing in mind that the harmful effects of aldosterone are not fully abolished by the MR blockade, since alternative receptors (GR, GPR30, AT_1), as well as nongenomic mechanisms are involved in the hormone action, the question arises whether blocking at the level of aldosterone synthesis would be more beneficial in this case (Figure 2).

The key enzyme in aldosterone production is aldosterone synthase (CYP11B2). CYP11B2 is predominantly expressed in the adrenal gland but is also expressed in the cardiovascular system or brain.[109–111] Lack of optimal effectiveness in aldosterone receptor blockade initiated some research on ASIs, like FAD286 or LCI699.[112]

FAD286

FAD286, the R-enantiomer of fadrozole, was initially developed as an aromatase (CYP19A1) inhibitor and used as a drug to treat breast cancer.[111] There were also demonstrated potential benefits of FAD286 in the therapy of cardiovascular disorders in different experimental models of CVD and diabetes (Table 1).[110,113–121] Some effects were similar to the effects of MRAs, proving that aldosterone plays a key role in the pathogenesis of CVD. Considering that aldosterone may also act through the MR-independent pathways, ASIs seem to be an excellent supplement of classic MRAs therapy in the prevention of cardiac insufficiency. The results of experimental studies are promising, which allow us to believe that inhibition of aldosterone synthesis can support treatment of CVD.

LCI699

Following the experimental studies with FAD286, LCI699 was synthesized, based on the chemical structure of FAD286, as the first orally active ASI for human use (Table 2).[122] LCI699 is a potent inhibitor of CYP11B2, but it also inhibits CYP11B1, the enzyme that catalyses the final step of cortisol synthesis. The results of phase II studies showed that in patients with primary hyperaldosteronism characterized by severe hypertension and hypokalemia LCI699 induced a reversible and dose-dependent 70–80% decrease in plasma and urinary aldosterone concentrations with a massive accumulation of the aldosterone precursor, deoxycorticosterone, in the plasma, confirming the inhibition of the product of the CYP11B2 gene. Treatment with LCI699 caused correction of hypokalemia and a mild decrease in blood pressure.[123] The efficacy of LCI699 for lowering BP was investigated in patients with essential hypertension. The antihypertensive effect of 1 mg of LCI699 was similar to that of eplerenone at a dose of 50 mg.[124]

However, the effects of LCI699 on the glucocorticoid axis limit the use of higher doses because of the loss of selectivity for CYP11B2.[122] These effects on the glucocorticoid axis may not be a problem in the case of Cushing disease patients. In fact, preliminary results from a multicenter, proof-of-concept study are that patients with Cushing disease

achieve normal urinary cortisol with LCI699.[125] Another LCI699 trial goal was to evaluate the effect of LCI699 on cortisol response to adrenocorticotropic hormone stimulation in patients with essential hypertension in order to find the maximally tolerated dose in this patient population, which was estimated to be 1.30 mg once daily. The treatment was well tolerated with no serious adverse effects.[126] In a trial comparing LCI699 to eplerenone in 14 patients with primary aldosteronism, the effects on blood pressure and plasma potassium and renin concentrations of four weeks of eplerenone treatment were more significant than those of four weeks of LCI699 treatment, with the opposite drugs effect on plasma aldosterone concentrations.[127]

Other ASIs

The new inhibitors of CYP11B2 are already existing drugs that according to some researchers could be used either in the treatment of hyperaldosteronism-related diseases or as precursors to achieve safer and selective new ASIs.[128] Moreover, several dihydropyridine Ca^{2+} channel blockers block T-type channel as well, which brings upon the inhibition of aldosterone synthesis *in vitro*. The dihydropiridine structure might be the base for the development of novel molecules that dually (a) block aldosterone synthase and MR for more potent aldosterone antagonism and (b) inhibit the L-type Ca^{2+} channel for more pronounced antihypertensive effects.[129]

Combined treatment—another approach to RAAS blocking

The another approach to effective treatment of CVD is the usage of new combinations of agents modulating the RAAS. There are many clinical studies (RESOLVD, CHARM, ALOFT) showing the efficacy of dual RAAS blockade based on combination of various doses of well-known ACE-Is, ARBs, and direct renin inhibitor. Unfortunately, several clinical trials (ONTARGET, ALTITUDE and VA NEPHRON-D) in patients with hypertension, heart failure, and chronic kidney disease with proteinuria have demonstrated no beneficial effects of dual versus single RAAS blockade, but a higher incidence of adverse events.[130] Some new combined agents affecting RAAS occurred recently. According to the latest network meta-analysis of Xie et al., ARNI, a novel dual-acting angiotensin receptor-neprilysin inhibitor has the highest probability of being the most effective therapy for heart failure and reduced ejection fraction compared to ACE-Is and/or ARBs.[131]

Conclusions and perspectives

The efficacy of classic RAAS affecting drugs in CVD is widely known, but previously it was not assumed these effects could also be related with the activation of other regulatory elements of RAAS. Understanding the mechanism of new molecules' action in the RAAS allows the introduction of alternative therapies and thus elimination of the adverse effects of already used drugs. The emergence of these novel drugs may not only help to improve the effectiveness of treatment of CVD, but it may further significantly broaden the therapeutic potential of the RAAS. The results of basic experiments and clinical studies are encouraging, which leads us to believe that new molecules can support treatment of CVD and could be helpful in the management of cardiometabolic disorders.

More detailed information about the results of experimental studies with the usage of new agents affecting RAAS are enclosed in supplementary files (Supplementary Tables 1–3).

Author contributions: All authors contributed to the writing of this review article and have read and approved the final manuscript. The overall layout and content, preparation of figures and writing of the manuscript was carried out by AG-P. The survey of the literature regarding experimental studies with the use of agents modulating RAAS and summary tables were performed by PS and PK. The survey of the literature regarding clinical studies and part of writing was performed by KK and MW-Z. The part of the writing and revision of the manuscript was carried out by EC.

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DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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