Hypertension Research
https://doi.org/10.1038/s41440-020-0458-5

ARTICLE

Impact of mineralocorticoid receptor blockade with direct renin inhibition in angiotensin II-dependent hypertensive mice

Atsushi Hashimoto[1] · Yoshimichi Takeda[1] · Shigehiro Karashima[2] · Mitsuhiro Kometani[2] · Daisuke Aono[2] · Masashi Demura[3] · Takuya Higashitani[2] · Seigo Konishi[2] · Takashi Yoneda[4] · Yoshiyu Takeda[1,5]

Received: 18 September 2019 / Revised: 31 March 2020 / Accepted: 2 April 2020

Abstract

It has been suggested that aldosterone breakthrough during treatment with a type 1 angiotensin II receptor (AT1R) blocker (ARB) may be an important risk factor for the progression of renal and cardiovascular disease. We examined whether the direct renin inhibitor, aliskiren caused aldosterone breakthrough in angiotensin II (Ang II)-dependent hypertensive mice. The effect of combination therapy with aliskiren and eplerenone was compared with that of therapy using renin–angiotensin system (RAS) blockade. Tsukuba hypertensive mice were treated for 12 weeks with aliskiren (30 mg/kg/day, i.p), candesartan (5 mg/kg/day, p.o), eplerenone (100 mg/kg/day, p.o) aliskiren and candesartan, aliskiren and eplerenone or candesartan and eplerenone. Blood pressure, urinary aldosterone and angiotensinogen (AGTN) excretion; plasma endothelin-1 concentration; kidney weight; urinary albumin excretion (UAE); glomerular injury; and renal messenger RNA (mRNA) levels for transforming growth factor (TGF)-β1, plasminogen activator inhibitor (PAI)-1, angiotensin-converting enzyme (ACE) and AT1R were measured. Combination therapy with aliskiren and candesartan caused a further decrease in blood pressure ($p < 0.05$) compared with either agent alone. Urinary aldosterone excretion was decreased significantly by 4 weeks of treatment with aliskiren or candesartan ($p < 0.05$). However, it was increased again by treatment with candesartan or aliskiren for 12 weeks. Combination therapy with aliskiren and eplerenone significantly decreased UAE, the glomerulosclerosis index, and PAI-1 and TGF-β1 mRNA levels compared with all other therapies ($p < 0.05$). Treatment with aliskiren decreased urinary aldosterone excretion at 4 weeks and increased it at 12 weeks. Combination therapy with a direct renin inhibitor and a mineralocorticoid receptor blocker may be effective for the prevention of renal injury in Ang II-dependent hypertension.

Keywords Aldosterone · Renin inhibitor · Mineralocorticoid receptor blocker · Kidney · Hypertension

Introduction

Aldosterone plays an important role in the pathogenesis of renal and cardiovascular disease independent of the effects of angiotensin II (Ang II). Clinical studies have shown that mineralocorticoid receptor blockade therapy improves renal function in patients with hypertension or diabetes mellitus [1, 2], whereas data from experimental animal models also support a role for aldosterone in mediating renal injury [3, 4]. We have reported that the blockade of mineralocorticoid receptors (MRs) with eplerenone decreased the renal renin–angiotensin–aldosterone system (RAAS) [5]. These changes occurred concomitantly with an improvement in renal injury in salt-sensitive hypertensive rats. Aldosterone breakthrough during treatment with a type 1 angiotensin II receptor blocker (ARB) or angiotensin I converting enzyme inhibitor (ACEI) has been suggested as

✉ Yoshiyu Takeda
takeday@med.kanazawa-u.ac.jp

1 Department of Cardiovascular Medicine, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

2 Endocrinology and Metabolism, Kanazawa University Hospital, Kanazawa, Japan

3 Department of Hygiene, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

4 Institute of Liberal Arts and Science, Kanazawa University, Kanazawa, Japan

5 Innovative Clinical Research Center, Kanazawa University, Kanazawa, Japan

Published online: 12 May 2020

an important risk factor for the progression of renal and cardiovascular disease [6, 7]. The direct renin inhibitor (DRI), aliskiren, by suppressing the RAAS proximally, may limit aldosterone breakthrough compared with conventional therapy using an ACEI or an ARB. However, Sato et al. [8] observed that aldosterone breakthrough occurred in 55% of hypertensive patients with increased urinary albumin excretion during treatment with a DRI. Tsukuba hypertensive mice (THM) are transgenic mice that carry both human renin and angiotensinogen (AGTN) genes. These mice are prone to developing hypertension [9] with Kai et al. [10] reporting that albuminuria and glomerular sclerosis also occurred in these animals.

We examined whether aliskiren caused aldosterone breakthrough in Ang II-dependent hypertensive mice. The effect of combination therapy with aliskiren and the MR blocker (MRB), eplerenone was compared with that of conventional therapy using RAS blockade.

Methods

Animal experiments

All the experiments were performed according to the guidelines for the use of experimental animals of the Animal Research Committee of Kanazawa University. Male THM were purchased from RIKEN BioResource Center (Tsukuba, Japan) with the support of the National Bio-Resource Project of the Ministry of Education, Culture, Sports, Science and Technology of Japan. The THMs were divided into seven treatment groups: aliskiren (50 mg/kg/day, administered using subcutaneous implantation of an osmotic mini pump (Alzet), Novartis Switzerland), candesartan (5 mg/kg/day, p.o. Takeda Chemical Industries, Japan), eplerenone (100 mg/kg/ day, p.o. Pfizer USA), aliskiren and candesartan, aliskiren and eplerenone, candesartan and eplerenone or a control untreated group. Each group consisted of six THMs. Data collection began when the mice reached 10 weeks of age. The mice were treated with the drugs for 12 weeks and sacrificed at 22 weeks of age. All mice were housed in metabolic cages for the measurement of daily urinary excretion. BP was determined by the tail-cuff method using photoelectric volume oscillometry (BP-98A; Softron, Tokyo, Japan). In several mice, intra-arterial BP was measured as described previously [11]. The BP data measured by the tail-cuff method paralleled the data obtained from the direct intra-arterial measurements. Urinary aldosterone excretion was determined by radioimmunoassay following extraction using a Sep-Pak C18 cartridge (Nihon Waters K.K, Tokyo Japan) as reported previously [12]. Urinary aldosterone excretion is a good marker to estimate aldosterone synthesis of the adrenal gland. Urinary albumin and AGTN were measured using commercial ELISA kits. Plasma endothelin-1 was measured as reported previously [13].

Quantification of mRNA expression of plasminogen activator inhibitor-1, transforming growth factor-beta1, angiotensin-converting enzyme, and Ang II type 1 receptor in the kidney

Before the animals were sacrificed, they were anesthetized with pentobarbital (100 mg/kg, intraperitoneally), intubated and then mechanically ventilated. The abdomen was opened, blood was collected, and the kidneys were removed and weighed. Total RNA was extracted from the kidney using TRIzol (Invitrogen) according to the manufacturer's protocol. A real-time quantitative reverse transcription-polymerase chain reaction was carried out using the TaqMan One-Step RT-PCR Master Mix Reagent Kit and ABI Prism 7000 HT Detection System (Applied Biosystems, Tokyo, Japan) according to the manufacturer's protocol. The sequences of sense and antisense primers and probes for plasminogen activator inhibitor-1 (PAI-1), transforming growth factor-β1 (TGF-β1), AGTN, ACE, and AT1R were designed as reported previously [14, 15]. Serial dilutions of stock standard RNA were used to obtain a calibration curve. The relative amount of each messenger RNA (mRNA) was normalized to the housekeeping gene, 18S ribosomal RNA.

Histological analysis

Renal sections embedded in paraffin (3-mm thick) were stained with hematoxylin and eosin and examined by light microscopy. The semiquantitative glomerulosclerosis index was calculated by examining 100 glomeruli per section [16]. All morphometric measurements were performed by two examiners who had no knowledge of the treatment protocol.

Statistical analysis

Data are expressed as the mean ± SEM. Data were compared by a two-way analysis of variance (ANOVA) or Friedman's test and when each ANOVA indicated significance, Fisher's protected least significance or Scheffe's F test was performed. Statistical significance was inferred for $P < 0.05$.

Results

Figure 1 shows the effect of aliskiren, candesartan, eplerenone, and combination therapy with either aliskiren and candesartan or aliskiren and eplerenone on systolic blood

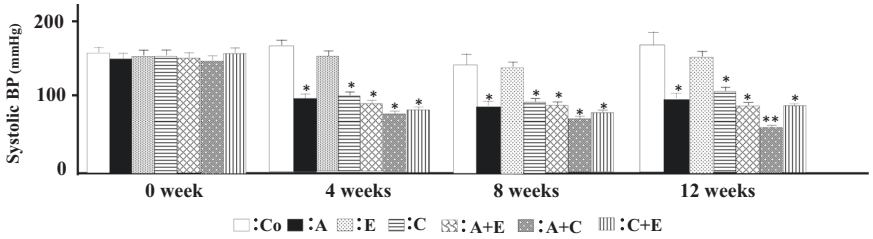

Fig. 1 Effect of aliskiren (A), candesartan (C), eplerenone (E), and their combination with A and C, A and E, or C and E on blood pressure in Tsukuba hypertensive mice. Control mice (Co) were not treated with an antihypertensive drug. *$p < 0.05$ vs pretreatment; **$p < 0.01$ vs pretreatment

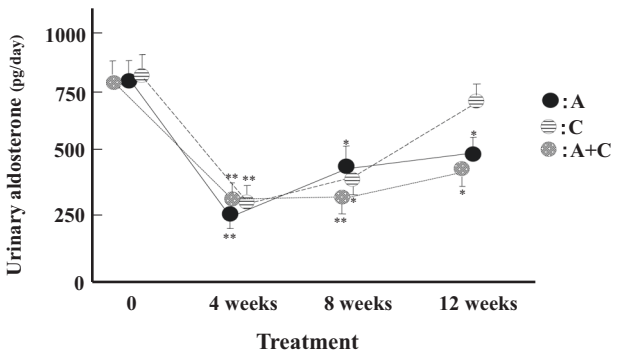

Fig. 2 Effect of aliskiren (A), candesartan (C), and their combination with A and C on urinary aldosterone excretion. *$p < 0.05$ vs pretreatment; **$p < 0.01$ vs pretreatment

pressure in THM. With the exception of eplerenone the drugs decreased blood pressure equally. Combination therapy with aliskiren and candesartan caused a further decrease in blood pressure. Treatment with aliskiren, candesartan or aliskiren and candesartan significantly decreased urinary aldosterone excretion at 4 weeks of treatment (Fig. 2). Urinary aldosterone excretion was increased further by treatment with candesartan at 12 weeks (Fig. 2). Treatment with aliskiren increased urinary aldosterone excretion with the levels being significantly lower than those with pretreatment. Urinary AGTN excretion was decreased significantly by treatment with all the agents ($p < 0.05$) (Fig. 3). Combination therapy with aliskiren and eplerenone decreased UAE significantly compared with that observed with all the other agents ($p < 0.05$) (Fig. 3). Serum creatinine and plasma endothlin-1 concentrations did not differ among the experimental groups (Table 1). Treatment with eplerenone increased serum potassium but not significantly. Kidney weight (mg)/body weight (g) was 8.2 ± 0.6 (vehicle), 6.2 ± 0.3 (aliskiren), 6.8 ± 0.3 (candesartan), 5.5 ± 0.2 (eplerenone), 7.2 ± 0.7 (aliskiren + candesartan), 5.7 ± 0.1 (aliskiren + eplerenone) and 6.0 ± 0.2 (candesartan + eplerenone). The agents decreased kidney weight equally ($p < 0.05$). Figure 4 shows the data of the glomerulosclerosis index and renal mRNA levels of PAI-1 and TGF-β1 for each experimental group. Treatment with aliskiren, candesartan, or eplerenone decreased the glomerulosclerosis index significantly ($P < 0.05$), with this decrease being greater with combination therapy of aliskiren and eplerenone than with the other combination therapies. The gene expression levels of PAI-1 and TGF-β1 were decreased significantly in each experimental group ($p < 0.05$). Treatment with aliskiren and eplerenone further decreased the expression of both genes. The expression of ACE and AT1R mRNA in the kidney did not differ among all treatment groups (data not shown).

Discussion

This study showed that treatment with aliskiren or candesartan decreased BP and that combination therapy with the two drugs further decreased BP. However, eplerenone did not decrease blood pressure. The hypotensive effect of eplerenone was reported in Dahl salt-sensitive hypertensive rats [5]. Endeman et al. [17] reported that eplerenone decreased blood pressure in salt-loaded stroke-prone spontaneously hypertensive rats (SHRSPs) but did not influence normal- or low- salt-loaded SHRSPs. Sakurabayashi-Kitade et al. [18] also reported that spironolactone did not decrease blood pressure but restored vascular remodeling in THM. The hypotensive effect of MRB may depend on the kind of experimental animals.

Aldosterone breakthrough is observed during ARB or ACEI treatment and is independent of blood pressure. We have reported that aldosterone might contribute directly to the progression of renal and cardiac injury through multiple mechanisms [6]. Increased local aldosterone synthesis has been reported in rat hypertensive hearts [19] and failed hearts [20]. Both animal and human studies have shown improved renal and cardiac structure and function with the MRB treatment of independent of circulating RAS [5, 21]. Naruse et al. [22] reported aldosterone breakthrough during AT1R therapy in SHRSPs. There is evidence that aliskiren, alone or in combination with an ARB, did not reduce the incidence of aldosterone breakthrough in patients with hypertension and proteinuria or albuminuria compared with

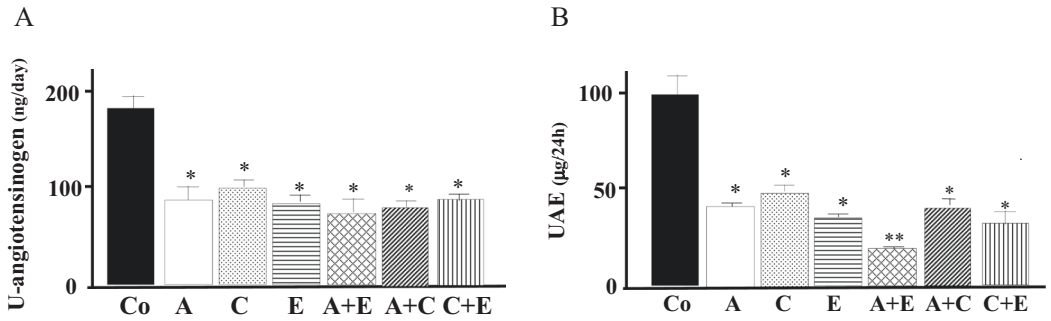

Fig. 3 Effect of aliskiren (A), candesartan (C), eplerenone (E), and their combination with A and C, A and E, or C and E on urinary excretion of albumin (3A) and angiotensinogen (3B) in Tsukuba hypertensive mice. Control mice (Co) were not treated with an antihypertensive drug. $*p < 0.05$ vs pretreatment; $**p < 0.01$ vs pretreatment

Table 1 Serum potassium (s-K), serum creatinine (s-Cr), and plasma endothelin-1 concentration (p-ET1) in the experimental groups

	Co	A	C	E	A + E	A + C	C + E
s-K (mEq/L)	4.1 ± 1.7	4.1 ± 1.7	4.1 ± 1.7	4.4 ± 1.8	4.3 ± 1.8	4.1 ± 1.7	4.3 ± 1.8
s-Cr (mg/dL)	0.11 ± 0.006	0.10 ± 0.007	0.11 ± 0.007	0.11 ± 0.005	0.11 ± 0.005	0.11 ± 0.006	0.10 ± 0.006
p-ET1 (pg/mL)	2.3 ± 0.3	2.5 ± 0.2	2.2 ± 0.1	2.3 ± 0.3	1.8 ± 0.2	2.2 ± 0.1	2.3 ± 0.2

Co control mice, *A* mice treated with aliskiren, *C* mice treated with candesartan, *E* mice treated with eplerenone, *A + E* mice treated with a combination of aliskiren and eplerenone, *A + C* mice treated with a combination of aliskiren and candesartan, *C + E* mice treated with a combination of candesartan and eplerenone

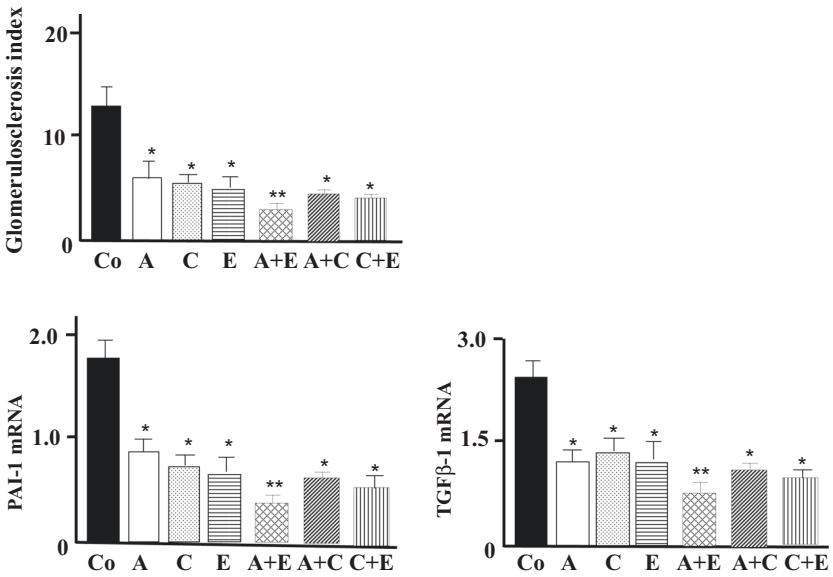

Fig. 4 Effect of aliskiren (A), candesartan (C), eplerenone (E), and their combination with A and C, A and E, or C and E on the glomerulosclerosis index, PAI-1 mRNA and TGF−β1 mRNA levels in Tsukuba hypertensive mice. Control mice (Co) were not treated with an antihypertensive drug. $*p < 0.05$ vs pretreatment; $**p < 0.01$ vs pretreatment

that observed with conventional RAS blockade [8, 23]. Our data showed that urinary aldosterone excretion was decreased over 4 weeks by treatment with aliskiren or candesartan. Treatment with candesartan further increased urinary aldosterone excretion at 12 weeks. Aliskiren or combination therapy of aliskiren with candesartan increased urinary aldosterone excretion at 12 weeks compared to that observed at 4 weeks.

Aldosterone synthesis is regulated by endothelin and atrial natriuretic peptide as well as Ang II and potassium. Rossi proposed the potential usefulness of endothelin antagonists for the prevention of aldosterone breakthrough [24]. In our study, treatment with aliskiren or an ARB did not influence plasma endothelin-1 concentration. Miura et al. [25] reported that the valsartan-neprilysin inhibitor LCZ696 blocked aldosterone synthesis in a human adrenocortical cell line. Aliskiren decreases plasma renin activity, Ang I, and Ang II. Renin or angiotensin I may not influence aldosterone breakthrough during treatment with aliskiren.

Urinary AGTN is a biomarker of acute and chronic renal injury [26]. We have reported that renal AGTN is a marker of intrarenal RAS activity in salt-sensitive hypertensive rats [5]. In the current study, UAE and urinary AGTN excretion were decreased in all the treatment groups. The normalization of albuminuria with the blockade of the RAAS is known to be a key therapeutic strategy for reducing the risk of renal and cardiovascular events in patients with hypertension [27]. Because increased RAS activity has been reported to play an important role in the hemodynamic and nonhemodynamic mechanisms involved in kidney injury, both ARBs and aliskiren have the potential to prevent the progression of nephropathy in a blood pressure-independent manner. Yoshida et al. [28] showed that aliskiren exhibited even stronger renoprotection than ramipril and they suggested aliskiren treatment resulted in lower Ang II concentration than ramipril. Uzu et al. [29] reported that both aliskiren and ARBs caused significant reductions in UAE in hypertensive patients with type 2 diabetes with the magnitude of this reduction being similar in the two treatment arms. They also reported that urinary AGTN excretion was significantly reduced in the ARB arm but not in the DRI arm, despite the BP control level being similar in both arms.

In our study, treatment with aliskiren and eplerenone further decreased UAE, the glomerulosclerosis index and gene expression of PAI-1 and TGF-β1 in the kidney. Kawarazaki et al. [30] using Ang II-overproducing double transgenic THM, a model of enhanced RAAS, demonstrated that Ang II/salt accelerates kidney injury by MR activation rather than via the Ang II receptor-mediated pathway. Renoprotective effects of MRBs through the inhibition of podocyte injury were demonstrated by immunohistochemistry [31, 32]. This study has potential limitations. Immunohistochemical analysis for glomerulosclerosis or podocyte injury was not performed. Further study is necessary to clarify the mechanism of the renoprotective effects of MRBs.

De Mello [33] also reported a beneficial effect of combination therapy with aliskiren and spironolactone on cardiac remodeling in Ang II-dependent hypertensive rats. MRBs are effective for low to high renin hypertension [34]. Li et al. [35] recently reported that a novel nonsteroidal selective MRB, esaxerenone, had antihypertensive and renal protective effects in salt-dependent hypertensive mice with suppressed intrarenal renin activity. Therefore, a further large-scale study in clinical hypertensive patients with high renin levels on the potential beneficial effects of combination therapy with aliskiren MRBs is warranted.

Conclusions

Treatment with aliskiren decreased urinary aldosterone excretion at 4 weeks and increased it at 12 weeks. Combination therapy with a DRI and an MRB may be effective for the prevention of renal injury in Ang II-dependent hypertension.

Acknowledgements We are grateful to Ms Cheng Wu for assistance with the animal experiment.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Nishimoto M, Ohtsu H, Marumo T, Kawarazaki W, Ayuzawa N, Ueda K, et al. Mineralocorticoid receptor blockade suppresses dietary salt-induced ACEI/ARB-resistant albuminuria in non-diabetic hypertension: a sub-analysis of evaluate study. Hypertens Res. 2019;42:514–21.
2. Yoneda T, Takeda Y, Usukura M, Oda N, Takata H, Yamamoto Y et al. Aldosterone breakthrough during angiotensin II receptor blockade in hypertensive patients with diabetes mellitus. Am J Hypertens. 2007;20:1329–33.
3. Shibata S, Ishizawa K, Uchida S. Mineralocorticoid receptor as a therapeutic target in chronic kidney disease and hypertension. Hypertens Res. 2017;40:221–5.
4. Nishiyama A. Pathophysiological mechanisms of mineralocorticoid receptor-dependent cardiovascular and chronic kidney disease. Hypertens Res. 2019;42:293–300.
5. Zhu A, Yoneda T, Demura M, Karashima S, Usukura M, Yamagishi M, et al. Effect of mineralocorticoid receptor blockade on the renal renin-angiotensin system in Dahl salt-sensitive hypertensive rats. J Hypertens. 2009;27:800–5.
6. Takeda Y. Effects of eplerenone, a selective mineralocorticoid receptor antagonist, on clinical and experimental salt-sensitive hypertension. Hypertens Res. 2009;32:321–4.
7. Ruiz-Hurtado G, Ruilope LM. Cardiorenal protection during chronic renin-angiotensin-aldosterone system suppression: evidences and caveats. Eur Heart J Cardiovasc Pharmacother. 2015;1:126–31.
8. Sato A, Fukuda S. Effect of aldosterone breakthrough on albuminuria during treatment with a direct renin inhibitor and combined effect with a mineralocorticoid receptor antagonist. Hypertens Res. 2013;36:879–84.
9. Fukamizu A, Sugimura K, Takimoto E, Sugiyama F, Seo MS, Takahashi S, et al. Chimeric renin-angiotensin system demonstrates sustained increase in blood pressure of transgenic mice carrying both human renin and human angiotensinogen genes. J Biol Chem. 1993;268:11617–21.
10. Kai T, Sugimura K, Shimada S, Kurooka A, Ishikawa K. Renin-angiotensin system stimulates cardiac and renal disorders in Tsukuba hypertensive mice. Clin Exp Pharm Physiol. 1999;26:206–11.
11. Takeda Y, Zhu A, Yoneda T, Usukura M, Takata H, Yamagishi M. Effects of aldosterone and angiotensin II receptor blockade on cardiac angiotensinogen and angiotensin-converting enzyme 2 expression in Dahl salt-sensitive hypertensive rats. Am J Hypertens. 2007;20:1119–24.
12. Takeda Y, Yoneda T, Demura M, Furukawa K, Usukura M, Mabuchi H. 19-Noraldosterone in pregnancy-induced hypertension. Steroids. 2002;67:605–10.

13. Takeda Y, Inaba S, Furukawa K, Fujimura A, Miyamori I, Mabuchi H. Effects of chronic neutral endopeptidase inhibition in rats with cyclosporine-induced hypertension. J Hypertens. 2000;18:927–33.
14. Higashi K, Oda T, Kushiyama T, Hyodo T, Yamada M, Suzuki S, et al. Additive antifibrotic effects of pioglitazone and candesartan on experimental renal fibrosis in mice. Nephrology. 2010;15:327–35.
15. Reinhold SW, Krüger B, Barner C, Zoicas F, Kammerl MC, Hoffmann U, et al. Nephron-specific expression of components of the renin-angiotensin-aldosterone system in the mouse kidney. J Renin Angiotensin Aldosterone Syst. 2012;13:46–55.
16. Nagase M, Shibata S, Yoshida S, Nagase T, Gotoda T, Fujita T. Podocyte injury underlies the glomerulopathy of Dahl salt-hypertensive rats and is reversed by aldosterone blocker. Hypertension. 2006;47:1084–93.
17. Endemann DH, Touyz RM, Iglarz M, Savoia C, Sciffrin EL. Eplerenone prevents salt-induced vascular remodeling and cardiac fibrosis in stroke-prone spontaneously hypertensive rats. Hypertension. 2004;43:1252–7.
18. Sakurabayashi-Kitade S, Aoka Y, Nagashima H, Kasanuki H, Hagiwara N, Kawana M. Aldosterone blockade by Spironolactone improves the hypertensive vascular hypertrophy and remodeling in angiotensin II overproducing transgenic mice. Atherosclerosis. 2009;206:54–60.
19. Takeda Y, Yoneda T, Demura M, Miyamori I, Mabuchi H. Cardiac aldosterone production in genetically hypertensive rats. Hypertension. 2000;36:495–500.
20. Yoshimura M, Nakamura S, Ito T, Nakayama M, Harada E, Mizuno Y, et al. Expression of aldosterone synthase gene in failing human heart: quantitative analysis using modified real-time polymerase chain reaction. J Clin Endocrinol Metab. 2002;87:3936–40.
21. Pitt B, Zannad F, Remme W, Cody R, Castaigne A, Perez A, et al. The effect of spironolactone on motbility and mortality in patients with severe heart failure. N. Engl J Med. 1999;341:709–17.
22. Naruse M, Tanabe A, Sato A, Takagi S, Tsuchiya K, Imaki T, et al. Aldosterone breakthrough during angiotensin II receptor antagonist therapy in stroke-prone spontaneously hypertensive rats. Hypertension. 2002;40:28–33.
23. Bomback AS, Rekhtman Y, Klemmer PJ, Canetta PA, Radhakrishnan J, Appel GB. Aldosterone breakthrough during aliskiren, valsartan, and combination (aliskiren + valsartan) therapy. J Am Soc Hypertens. 2012;6:338–45.
24. Rossi GP, Albertin G, Neri G, Andreis PG, Hofmann S, Pessina AC et al. Endothelin-1 stimulates steroid secretion of human adrenocortical cells ex vivo via both ETA and ETB receptor subtypes. J Clin Endocrinol Metab. 1997;82:3445–9.
25. Miura SI, Suematsu Y, Matsuo Y, Tomita S, Nakayama A, Goto M, et al. The angiotensin II type 1 receptor – nephrilysin inhibitor LCZ696 blocked aldosterone synthesis in a human adrenocortical cell line. Hypertens Res. 2016;39:758–63.
26. Connor KL, Denby L. Urinary angiotensinogen as a biomarker for acute to chronic kidney injury transition - prognostic and mechanistic implications. Clin Sci. 2018;132:2383–5.
27. Emdin CA, Rahimi K, Neal B, Callender T, Perkovic V, Patel A. Blood pressure lowering in type 2 diabetes: a systematic review and meta-analysis. JAMA. 2015;13:603–15.
28. Yoshida S, Ishizawa K, Ayuzawa N, Ueda K, Takeuchi M, Kawarazaki W, et al. Renin inhibition ameliorates renal damage through prominent suppression of both angiotensin I and II in human renin angiotensinogen transgenic mice with high salt loading. Clin Exp Nephrol. 2014;18:593–9.
29. Uzu T, Araki SI, Kashiwagi A, Haneda M, Koya D, Yokoyama H, et al. Comparative effects of direct renin inhibitor and angiotensin receptor blocker on albuminuria in hypertensive patients with type 2 diabetes. A randomized controlled trial. PLoS ONE. 2016;29: e0164936. 11(12).
30. Kawarazaki W, Nagase M, Yoshida S, Takeuchi M, Ishizawa K, Ayuzawa N, et al. Angiotensin II- and salt-induced kidney injury through Rac1-mediated mineralocorticoid receptor activation. J Am Soc Nephrol. 2012;23:997–1007.
31. Nagase M, Matsui H, Shibata S, Gotoda T, Fujita T. Salt-induced nephropathy in obese spontaneously hypertensive rats via paradoxical activation of the mineralocorticoid receptor: role of oxidative stress. Hypertension. 2007;50:877–83.
32. Kiyomoto H, Rafiq K, Mostofa M, Nishiyama A. Possible underlying mechanisms responsible for aldosterone and mineralocorticoid receptor-dependent renal injury. J Pharm Sci. 2008; 108:399–405.
33. De Mello WC. Spironolactone enhances the beneficial effect of aliskiren on cardiac structural and electrical remodeling in TGR (mRen2)27 rats. J Renin Angiotensin Aldosterone Syst. 2015; 16:488–94.
34. Weinberger MH, White WB, Ruilope LM, MacDonald TM, Davidson RC, Roniker B, et al. Effects of eplerenone versus losartan in patients with low-renin hypertension. Am Heart J. 2005;150:426–33.
35. Li L, Guan Y, Kobori H, Morishita A, Kobara H, Masaki T, et al. Effects of the novel nonsteroidal mineralocorticoid receptor blocker, esaxerenone (CS-3150), on blood pressure and urinary angiotensinogen in low-renin Dahl salt-sensitive hypertensive rats. Hypertens Res. 2019;42:769–78.