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ARTICLE

# Greater reductions in plasma aldosterone with aliskiren in hypertensive patients with higher soluble (Pro)renin receptor level

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## Abstract

The (pro)renin receptor is important in the regulation of the tissue renin-angiotensin-aldosterone system. The benefits and safety of single-aliskiren treatment without other renin-angiotensin-aldosterone system inhibitors remain unclear. The serum level of the soluble form of the (pro)renin receptor is thought to be a biomarker reflecting the activity of the tissue renin-angiotensin-aldosterone system. We investigated the effects of single renin-angiotensin-aldosterone system blockade with aliskiren on renal and vascular functions and determined if serum level of the soluble (pro)renin receptor was a predictor of aliskiren efficacy in hypertensive patients with chronic kidney disease. Thirty-nine essential hypertensive patients with chronic kidney disease in our outpatient clinic were randomly assigned to receive either aliskiren or amlodipine. The parameters associated with renal and vascular functions and indices of renin-angiotensin-aldosterone system components, including serum levels of the soluble form, were evaluated before and after 12-week and 24-week treatment. Blood pressure was not significantly different between the groups. No significant changes in serum levels were observed in the soluble (pro) renin receptor in either group. Urinary albumin, protein excretion, and cardio-ankle vascular index significantly decreased in the aliskiren group. In the aliskiren group, there was a significant negative correlation between the basal level of the soluble (pro)renin receptor and the change in plasma aldosterone concentration. Single renin-angiotensin-aldosterone system blockade with aliskiren showed renal and vascular protective effects independent of blood pressure reduction. Serum levels of the soluble (pro)renin receptor may indicate aldosterone production via the (pro)renin receptor in the adrenal gland.

## Introduction

The pathophysiological roles of the (pro)renin receptor [(P)RR] are a subject of increasing interest. It binds to renin and prorenin, which leads to tissue angiotensin generation and angiotensin-independent activation of intracellular signaling. The soluble form of the (P)RR [s(P)RR] is secreted into the extracellular space and can be measured in human blood and urine samples; furthermore, it is supposed to be a biomarker of the activity of the tissue renin-angiotensin-aldosterone system (RAAS). We recently identified a negative correlation between s(P)RR and estimated glomerular filtration rate (eGFR) in hypertensive patients [1]. High-circulating levels of s(P)RR in pregnant women have also been associated with blood pressure (BP) elevation and preeclampsia [2]. Furthermore, population-based clinical studies have suggested that (P)RR gene polymorphisms may reflect BP levels [3]. It is possible that enhancement of the tissue RAAS via (P)RR in patients with chronic kidney disease (CKD) is associated with BP elevation and organ damage; thus, s(P)RR levels could predict the progression of CKD.

Aliskiren, a direct renin inhibitor, has been reported to affect (P)RR-mediated angiotensin II (AngII)-dependent action and (P)RR expression in experimental models [4]. Therefore, aliskiren may exert protective effects on organs through its interaction with (P)RR. However, its efficacy in the prevention of organ damage and safety when combined with other RAAS blocking agents remain questionable. The

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efficacy of aliskiren in the prevention of end-organ damage is under investigation in the ASPIRE HIGHER program [5, 6]. The ALTITUDE (Aliskiren Trial in Type 2 Diabetes Using Cardio-renal Disease Endpoints) [7] trial indicated that the combination of aliskiren and other RAAS blocking agents is not recommended for hypertensive patients with diabetes, owing to the risk of development of new adverse events. Data regarding the amelioration of organ damage and safety are lacking, but there may be a potential patient population that would receive greater benefit from aliskiren.

In this study, we examined the effects of a single RAAS blockade with aliskiren on renal and vascular functions and determined if serum s(P)RR level was a predictor of the efficacy of aliskiren in hypertensive patients with CKD.

## Methods

### Study population and design

We enrolled 44 hypertensive patients with CKD, who visited our outpatient clinic between April 2012 and September 2015, in this study. Five patients dropped out before the beginning of the study. For previously untreated and treated patients, BP at the time of the study examination was SBP 140–179 mmHg and/or DBP 90–109 mmHg, and SBP 140–159 mmHg and/or DBP 90–99 mmHg, respectively. All patients fulfilled the diagnostic criteria for CKD provided by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative [8]. Patients with eGFR $< 60$ mL/min/1.73 $m^2$ and/or urine albumin excretion (UAE) $\geq 30$ mg/gCr were included.

Patients were excluded from study participation if they met any of the exclusion criteria: younger than 21 years; CKD Grade 4 or above; hyperkalemia (≥5.5 mEq/L); secondary hypertension (endocrine hypertension and renovascular hypertension); unstable angina; onset of myocardial infarction or brain stroke within a 6-month period prior to study examination; severe liver dysfunction; pregnancy or lactation; allergies to aliskiren or amlodipine; receipt of antihypertensive drugs other than amlodipine, nifedipine, and doxazosin within a 4-week period prior to study examination; ineligibility judged by the principal investigator. Thirty-nine patients were randomly assigned to the aliskiren group (DRI group) or the amlodipine group (CCB group). In the DRI group, aliskiren treatment was started; in the CCB group, amlodipine was started, or for patients previously prescribed amlodipine, the dose was increased. Aliskiren was initially prescribed at a dose of 150 mg/day and subsequently increased to a maximum dose of 300 mg/day if the target office BP of <130/80 mmHg was not achieved. The dose of amlodipine was subsequently increased to a maximum dose of 10 mg/day if the target office BP of <130/80 mmHg was not achieved. If the target BP was not achieved by the maximum dose of each drug, additional treatment of doxazosin was started and the dose was subsequently increased to a maximum of 8 mg/day in both groups. Clinical and biological parameters were measured before and after the 12-week and 24-week treatment periods. For serum pentraxin 3 (PTX-3), urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG), cardio-ankle vascular index (CAVI), ankle brachial index (ABI), augmentation index (AI), central systolic BP (cBP), flow mediated dilation (FMD), and carotid intima-media thickness (IMT), two replicate measurements were recorded before and after the 24-week treatment period. The study was approved by the ethical review committee of Tokyo Women's Medical University Hospital (approval No. 2434) and written informed consent was obtained from every subject. The primary measure of the present study was the serum s(P)RR level.

### Background factors

At enrollment, information was collected on age, gender, body mass index (BMI), waist circumference, complications of impaired glucose tolerance (IGT) and dyslipidemia, smoking history, and family history of cardiovascular disease (CVD). Waist circumference was measured at the height of the umbilicus while the patient was standing and after exhaling.

### Office BP

Office BP was obtained at an outpatient clinic with the patient in a sitting position and after at least 5 min of rest. The first reading at each visit was used for this study.

### Blood examinations

Blood samples were drawn in the morning after an overnight fast, with the patient in a sitting position, and after at least 15 min of rest. Serum potassium (K), uric acid (UA), creatinine, cystatin C (cysC), fasting blood glucose (FBS), glycated hemoglobin (HbA1c), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), high-sensitivity C-reactive protein (hsCRP), and brain natriuretic peptide (BNP) were measured by standard laboratory methods at our clinical laboratory center. Serum s(P)RR levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit with highly specific antibodies for the protein (Takara Bio, Otsu City, Japan). Plasma renin concentration (PRC), plasma renin activity (PRA), and plasma aldosterone concentration (PAC) were measured by radioimmunoassay. Serum PTX-3, a marker for vascular inflammation, was

**Table 1** Patient characteristics at baseline

| | DRI ($n = 20$) | CCB ($n = 19$) | $p$-value |
|---|---|---|---|
| Age, years | 57 ± 12 | 60 ± 13 | 0.4093 |
| Male, $n$ (%) | 12 (60) | 8 (42) | 0.2638 |
| BMI, kg/m$^2$ | 25.8 ± 3.7 | 24.9 ± 4.9 | 0.5542 |
| Waist circumference, cm | 93.3 ± 7.7 | 91.4 ± 11.3 | 0.5721 |
| IGT, $n$ (%) | 6 (30) | 2 (11) | 0.1322 |
| Dyslipidemia, $n$ (%) | 7 (35) | 7 (36) | 0.9046 |
| Smoking, $n$ (%) | 3 (16) | 3 (20) | 0.7491 |
| FH of CVD, $n$ (%) | 1 (5) | 2 (13) | 0.3835 |
| Office BP, mmHg | | | |
| systolic | 148 ± 3 | 148 ± 16 | 0.9316 |
| diastolic | 91 ± 2 | 86 ± 3 | 0.169 |
| eGFR, mL/min/1.73 m$^2$ | 72.8 ± 16.7 | 73.0 ± 27.3 | 0.9783 |
| UAE, mg/gCr | 113.8 ± 41.7 | 128 ± 45.2 | 0.814 |
| UPE, g/gCr | 0.15 ± 0.19 | 0.20 ± 0.32 | 0.6064 |
| CysC, mg/L | 0.96 ± 0.25 | 0.91 ± 0.20 | 0.5199 |
| FBS, mg/dL | 112 ± 30 | 110 ± 22 | 0.7878 |
| Serum s(P)RR, ng/mL | 22.0 ± 4.6 | 22.3 ± 5.6 | 0.877 |
| PRC, pg/mL | 9.6 ± 11.2 | 10.9 ± 10.9 | 0.7367 |
| PRA, ng/mL/h | 1.5 ± 1.4 | 1.5 ± 1.2 | 0.9179 |
| PAC, pg/mL | 134 ± 58 | 161 ± 68 | 0.1928 |
| CAVI | 8.1 ± 0.9 | 8.3 ± 1.2 | 0.5424 |
| AI, % | 75 ± 13 | 78 ± 15 | 0.5202 |
| cBP, mmHg | 147 ± 15 | 151 ± 18 | 0.4899 |
| FMD, % | 4.7 ± 2.3 | 5.0 ± 2.4 | 0.7106 |
| Max IMT, mm | 1.1 ± 0.4 | 1.1 ± 0.5 | 0.8868 |

Data are mean ± standard deviation for continuous variables, number (percentage) for categorical variables

IGT impaired glucose tolerance, FH family history, CVD cardiovascular disease, BP blood pressure, eGFR estimated glomerular filtration ratio, UAE urine albumin excretion, UPE urine protein excretion, CysC cystatin C, FBS fasting blood glucose, s(P)RR soluble (pro)renin receptor, PRC plasma renin concentration, PRA plasma renin activity, PAC plasma aldosterone concentration, CAVI cardio-ankle vascular index, AI augmentation index, cBP central blood pressure, FMD flow mediated dilation, max IMT maximum intima-media thickness of the carotid arteries

measured by ELISA at an external laboratory (SRL, Tokyo, Japan). The estimated glomerular filtration rate (eGFR) was calculated by using the following equation:

$$\mathrm{eGFR(mL/min/1.73\ m^2)} = 194 \times \mathrm{creatinine} - 1.094 \times \mathrm{age} - 0.287(\times 0.739 \text{ if female})$$

## Urinary examinations

Spot urine samples were obtained and concentrations of creatinine, albumin, and protein were quantified by standardized assessment methods at our clinical laboratory center. 8-OHdG, a marker for oxidative stress, was measured by ELISA at an external laboratory (SRL, Tokyo, Japan). Excretory levels of albumin, protein, and 8-OHdG were evaluated by the division of the obtained values by the creatinine level.

## Cardio-ankle vascular index and ankle brachial index

CAVI and ABI were measured using a VaSera VS-1500AN vascular screening system (Fukuda Denshi, Tokyo, Japan), as previously described [9].

## Augmentation index and central systolic BPs

AI and cBP were measured using an automated tonometric device (HEM-9000AI; Omron Healthcare, Kyoto, Japan), as previously described [10, 11].

## Flow mediated dilation

Percentage changes in brachial artery diameter were calculated in response to increased FMD, an index of endothelial function, as previously described [12, 13] by using a UNEX EF38G (UNEX, Nagoya, Japan).

## Carotid intima-media thickness

Ultrasonography B-mode imaging of the carotid artery was performed with a Nemio XG ultrasound system (Toshiba, Tokyo, Japan) at a transducer frequency of 7.5 MHz, as previously described [1].

## Statistical analyses

Statistical analyses were performed by using JMP® Pro 12.1.0 software and SAS ver.9.4 (SAS Institute Inc, Cary, NC, USA). A paired $t$-test was used to analyze patient baseline characteristics, with the exceptions of sex, presence of IGT, dyslipidemia, smoking history, family history of CVD. The changes in parameters within group were analyzed by a paired $t$-test. Between-group difference in time transition was analyzed by an analysis of variance considering time dependency using generalized linear model procedure. An unpaired $t$-test and a multivariate analysis of variance were applied to compare two groups. The contribution of changes in DBP to UAE was tested by analysis of covariance. Single analysis was performed to determine the correlations between parameters. A $P$-value of $<0.05$ was considered significant. Data are reported as the mean ± SD.

**Table 2** Measures of other variables at baseline and after 12 and 24 weeks of treatment

| | DRI | | | | CCB | | | | $p$-value[a] |
|---|---|---|---|---|---|---|---|---|---|
| Treatment period, weeks | 0 | 12 | 24 | $p$-value[b] | 0 | 12 | 24 | $p$-value[b] | |
| serum K, mEq/L | 4.1 ± 0.2 | 4.1 ± 0.3 | 4.2 ± 0.3 | 0.215 | 4.2 ± 0.3 | 4.2 ± 0.4 | 4.2 ± 0.3 | 0.3761 | 0.4584 |
| UA, mg/dL | 5.8 ± 1.6 | 6.2 ± 1.6 | 6.1 ± 1.8 | 0.17 | 5.6 ± 1.5 | 5.5 ± 1.3 | 5.3 ± 1.3[b] | **0.0207** | 0.3112 |
| CysC, mg/L | 0.96 ± 0.25 | 0.95 ± 0.29 | 0.98 ± 0.3 | 0.0763 | 0.91 ± 0.2 | 0.89 ± 0.19 | 0.89 ± 0.20 | 0.1135 | 0.4112 |
| AI, % | 75 ± 13 | N/A | 72 ± 20 | 0.2618 | 78 ± 15 | N/A | 78 ± 15 | 0.2282 | 0.8813 |
| FMD, % | 4.7 ± 2.3 | N/A | 5.1 ± 3 | 0.2526 | 5.0 ± 2.4 | N/A | 4.7 ± 2 | 0.2767 | 0.1140 |
| max IMT, mm | 1.1 ± 0.4 | N/A | 1.3 ± 0.6 | 0.1388 | 1.1 ± 0.5 | N/A | 1.3 ± 0.9 | 0.3313 | 0.8975 |
| ABI | 1.14 ± 0.10 | N/A | 1.17 ± 0.06 | 0.8055 | 1.14 ± 0.05 | N/A | 1.14 ± 0.06 | 0.8756 | 0.9040 |
| HbA1c, % | 6.1 ±1.1 | 6.1 ± 1.1 | 6.1 ± 1.3 | 0.3113 | 5.9 ± 0.4 | 5.9 ± 0.4 | 5.8 ± 0.4 | 0.0865 | 0.3845 |
| HOMA-R | 4.2 ± 4.4 | 6.8 ± 8.7 | 6.7 ± 11.1 | 0.1857 | 7.7 ± 17.3 | 5.5 ± 7.7 | 2.7 ± 1.9 | 0.1882 | 0.9324 |
| TG, mg/dL | 153 ± 80 | 224 ± 191 | 165 ± 98 | 0.1541 | 125 ± 78 | 118 ± 56 | 116 ± 46 | 0.3794 | **0.0494** |
| LDL-C, mg/dL | 135 ± 37 | 124 ± 30 | 125 ± 30[b] | **0.0410** | 128 ± 25 | 126 ± 29 | 121 ± 30 | 0.0984 | 0.9702 |
| HDL-C, mg/dL | 55 ± 15* | 52 ± 14 | 55 ± 14 | 0.4758 | 68 ± 21 | 67 ± 18 | 68 ± 18 | 0.073 | 0.6301 |
| hsCRP, mg/dL | 1853 ± 4855 | 884 ± 1393 | 1326 ± 2328 | 0.3223 | 1464 ± 2195 | 760 ± 847 | 789 ± 905 | 0.1162 | 0.6289 |
| PTX-3, ng/mL | 1.28 ± 0.41 | N/A | 1.27 ± 0.46 | 0.9769 | 1.45 ± 0.75 | N/A | 2.19 ± 3.75 | 0.3978 | 0.3457 |
| 8-OHdG, ng/mgCr | 10.1 ± 3.2 | N/A | 10.6 ± 3.1 | 0.6195 | 10.2 ± 5.5 | N/A | 12.0 ± 5.8 | 0.2481 | 0.3152 |
| Plasma BNP,mg/dL | 18.3 ± 17.3 | 22.4 ± 35.6 | 15.4 ± 18.8 | 0.1252 | 31.3 ± 43.6 | 29.9 ± 35.9 | 32.9 ± 42.5 | 0.2885 | 0.2522 |

Data are mean±standard deviation

*N/A* not available, *UA* uric acid, *ABI* ankle brachial index, *HbA1c* glycated hemoglobin, *HOMA-R* homeostasis model assessment ratio, *TG* triglyceride, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *hsCRP* high-sensitivity C-reactive protein, *PTX-3* pentraxin 3, *8-OHdG* 8-hydroxy-2′-deoxyguanosine, *BNP* brain natriuretic peptide

[a] DRI group vs CCB group, assessed by analysis of variance considering time dependency. Bold values are those found to be significant; $p$-value < 0.05.

[b] Within-groups (0 wk vs 24 wk), assessed by paired $t$-test. Bold values are those found to be significant; $p$-value < 0.05.

*$p$-value < 0.05 for DRI group vs CCB group at 0 weeks

## Results

### Patient characteristics

The characteristics of the DRI and CCB groups at baseline and after 12-week and 24-week treatment are shown in Tables 1 and 2. The diagnostic criteria of CKD by eGRF, by UAE, and by both eGRF and UAE were met by 4, 13, and 3 patients in the DRI group and 6, 11, and 2 patients, in the CCB group, respectively. Except for HDL-C, which was significantly lower in the DRI group, there were no significant differences in baseline variables. The doses of aliskiren prescribed for the DRI group at the end of observation period were 150 mg/day in 9 patients and 300 mg/day in 10 patients; the average dose was 217.5 ± 90.7 mg/day. The doses of amlodipine prescribed for the CCB group were 2.5 mg/day in 3 patients, 5 mg/day in 8 patients, 7.5 mg/day in 1 patient, and 10 mg/day in 8 patients; the average dose was 6.6 ± 2.9 mg/day. Doxazosin was prescribed to 3 patients in the DRI group at an average dose of 1.7 ± 0.6 mg/day and to 3 patients in the CCB group at an average dose of 3.3 ± 1.2 mg/day.

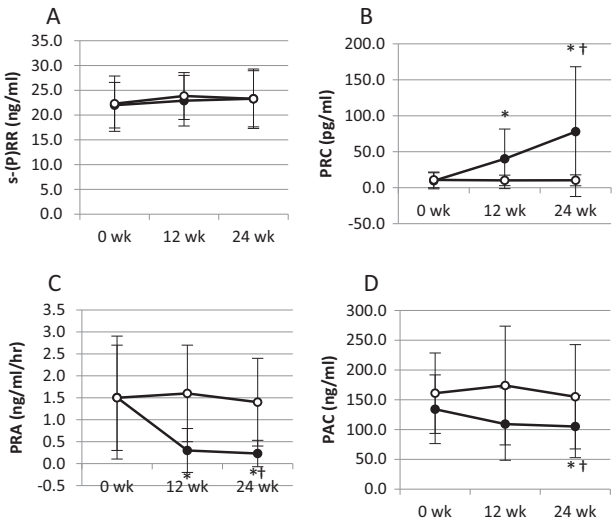


**Fig. 1** Serum soluble (pro)renin receptor [s(P)RR]) (**a**), plasma renin concentration (PRC) (**b**), plasma renin activity (PRA) (**c**), and plasma aldosterone concentration (PAC) (**d**) during 24-week treatment with aliskiren (closed circles, $n = 20$) and amlodipine (open circles, $n = 19$). *$p < 0.05$ vs 0 weeks, assessed by paired $t$-test, †$p < 0.05$ vs CCB, assessed by analysis of variance considering time dependency

## RAAS components

The changes in serum s(P)RR level, PRC, PRA, and PAC during the treatment period are shown in Fig. 1. The s(P)RR level remained unchanged both in the DRI group (22.0 ± 4.6 ng/mL at 0 weeks, 22.9 ± 5.1 ng/mL at 12 weeks, and 23.3 ± 6.0 ng/mL at 24 weeks) and in the CCB group (22.3 ± 5.6 ng/mL at 0 weeks, 24.5 ± 5.3 ng/mL at 12 weeks, and 24.3 ± 5.4 ng/mL at 24 weeks) (Fig. 1a). PRC significantly increased after treatment in the DRI group, but remained unchanged in the CCB group (Fig. 1b). A significant decrease in PRA was observed in the DRI group, but PRA remained unchanged in the CCB group (Fig. 1c). PAC significantly decreased after 24-week treatment period in the DRI group and remained unchanged in the CCB group (Fig. 1d).

## Office BP and cBP

The office BP and cBP at baseline and after 12-week and 24-week treatment are shown in Fig. 2. Systolic office BP decreased significantly after the treatment by comparable amounts in both groups (Fig. 2a). Diastolic office BP decreased significantly in the DRI group, but the decrease in the CCB group at 24 weeks was not significant. In contrast, cBP was unchanged in the DRI group, but a significant reduction was observed in the CCB group (Fig. 2b). However, when assessed by a multivariate repeated-measures approach, the changes in diastolic BP and cBP were comparable between the two groups.

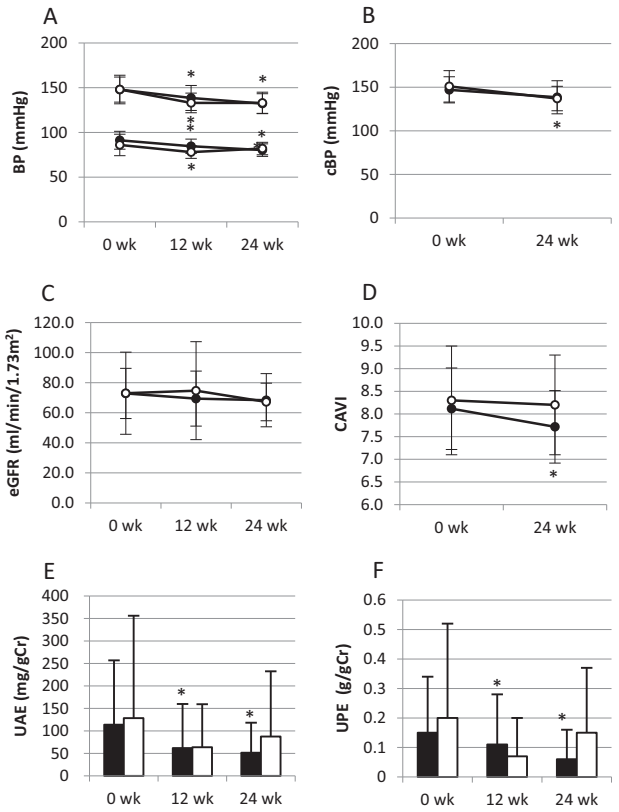


**Fig. 2** Office blood pressure (BP) (**a**), central blood pressure (cBP) (**b**), estimated glomerular filtration rate (eGFR) (**c**), cardio-ankle vascular index (CAVI) (**d**), urine albumin excretion (UAE) (**e**), and urine protein excretion (UPE) (**f**) during 24-week treatment with aliskiren (closed circles and bars, $n = 20$) and amlodipine (open circles and bars, $n = 19$). *$p < 0.05$ vs 0 weeks, assessed by paired $t$-test, $^{\dagger}p < 0.05$ vs CCB, assessed by analysis of variance considering time dependency

## Parameters of renal function

The values of eGFR, UAE, and urine protein excretion (UPE) at baseline and 12 and 24 weeks after treatment are shown in Fig. 2. There was no change in eGFR during treatment in both the groups (Fig. 2c). A significant reduction in UAE was observed after the treatment with DRI (113.8 ± 143 mg/gCr at 0 weeks, 61.8 ± 98.1 mg/gCr at 12 weeks, and 51.5 ± 66.7 mg/gCr at 24 weeks), but not with CCB (128.4 ± 227.7 mg/gCr at 0 weeks, 63.7 ± 95.6 mg/gCr at 12 weeks, and 87.5 ± 145.1 mg/gCr at 24 weeks) (Fig. 2e). UPE also decreased significantly after treatment in the DRI group (0.15 ± 0.19 g/gCr at 0 weeks, 0.11 ± 0.17 g/gCr at 12 weeks, and 0.06 ± 0.10 g/gCr at 24 weeks), but not in the CCB group (0.20 ± 0.32 g/gCr at 0 weeks, 0.07 ± 0.13 g/gCr at 12 weeks, and 0.15 ± 0.22 g/gCr at 24 weeks) (Fig. 2f). The serum level of UA significantly decreased after treatment in CCB group, but not in the DRI group. However, there was no between-group difference. The serum levels of K and cysC remained unchanged during the study period in both groups (Table 2).

## Evaluation of arterial stiffness and morphology

The changes in CAVI are shown in Fig. 2d. CAVI decreased significantly after the 24-week treatment period in the DRI group (8.1 ± 0.9 at 0 weeks and 7.7 ± 0.8 at 24 weeks), but remained unchanged in the CCB group (8.3 ± 1.2 at 0 weeks and 8.2 ± 1.1 at 24 weeks). However, there was no significant between-group difference in time transition. AI, FMD, max IMT, and ABI remained unchanged in both groups (Table 2).

## Parameters of metabolism, inflammation, oxidative stress, and cardiac function

The changes in metabolic parameters are shown in Table 2. Although there were no significant changes in TG levels with both treatments, significant between-group difference in time transition was observed. LDL-C significantly decreased after treatment in DRI group, but remained unchanged in CCB group. However, there was no between-group difference. HbA1c, HOMA-R, and HDL-C were

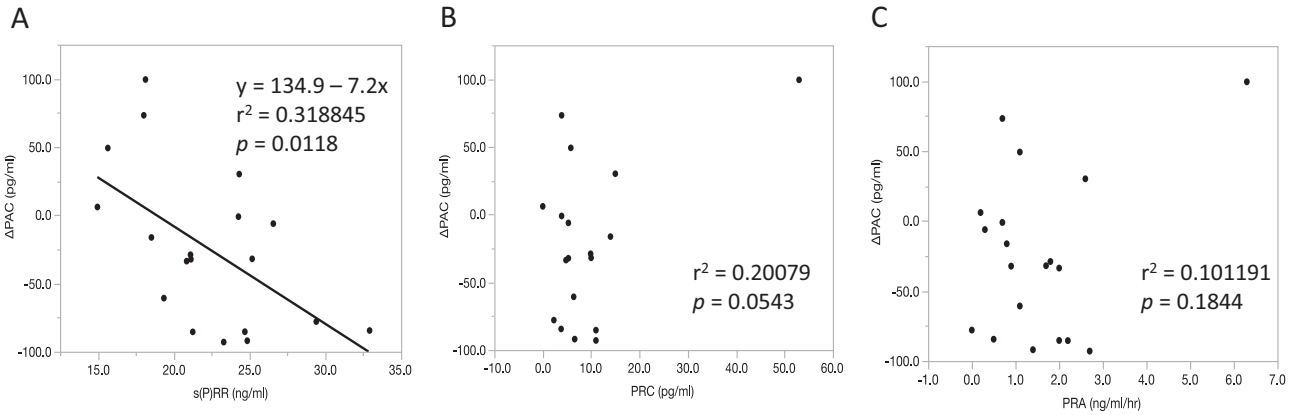


**Fig. 3** Scatter diagrams of the change in plasma aldosterone concentration (ΔPAC) after the 24-week treatment period and baseline serum soluble (pro)renin receptor [s(P)RR] (**a**), plasma renin concentration (PRC) (**b**), plasma renin activity (PRA) (**c**) of DRI group

unchanged during the treatment period. The values of hsCRP, PTX-3, 8-OHdG, and plasma BNP are also shown in Table 2; these remained unchanged during the observation period.

### Relationship between s(P)RR and the changes in RAAS components

To investigate if the basal s(P)RR level predicted the changes in other RAAS components, BPs, and markers of arterial stiffness and renal function, we examined the correlations between baseline serum s(P)RR level and the changes in these factors. In the DRI group, there was a significant negative correlation between serum s(P)RR and the change in PAC (Fig. 3a, $r^2 = 0.319$, $p = 0.01$), which was not observed in the CCB group (data not shown). In both groups, changes in PAC were not correlated with basal PRC and PRA (DRI group; Fig. 3b, c, CCB group; data not shown). Baseline serum s(P)RR was not correlated with basal PRC or PRA (Fig. 4a, b) and basal s(P)RR was not correlated with changes in other parameters in either group (data not shown).

## Discussion

The present study demonstrated two major findings. First, single RAAS blockade with aliskiren conferred superior organ protection compared with that of a single treatment of amlodipine. Second, in the patients treated with aliskiren, basal serum s(P)RR level was negatively correlated with the change in PAC.

In the DRI group, but not in the CCB group, significant reductions in UAE and UPE were observed after treatment (Fig. 2e, f). The renoprotective effect of aliskiren, independent of BP, has been reported previously in several studies [14, 15]. As the significant reduction in DBP was only observed in the DRI group, the reduction in DBP might have influenced UAE and UPE. However, there was no correlation between ΔDBP and either ΔUAE or ΔUPE, which suggested that UAE and UPE decreased independently of DBP reduction.

CAVI provides information on arterial stiffness and is a significant predictor of CVD [16, 17]. CAVI decreased significantly after the 24-week treatment in the DRI group, but was unchanged in the CCB group (Fig. 2d). Although the effect of aliskiren on CAVI has not been previously evaluated, the beneficial effect of aliskiren on endothelial function and other arterial stiffness indices has been demonstrated in several previous studies [18]. In these studies, AI, FMD, and IMT of the carotid artery were improved by aliskiren, which was contradictory to the results of our present study. Similar to UAE and UPE, there was no correlation between ΔDBP and ΔCAVI, which indicated that the decrease in DBP caused by aliskiren did not contribute to the reduction in CAVI.

Although the change was not significant, PAC tended to decrease after treatment in the DRI group and ΔPAC was significantly correlated to basal s(P)RR level (Fig. 3a). To elucidate the mechanism underlying this phenomenon, we analyzed the association of ΔPAC with other RAAS components. ΔPAC did not correlate with basal PRC or PRA, which are the indicators of circulatory RAAS activity. We also determined if s(P)RR was associated with PRC and PRA; however, there were no correlations between basal s(P)RR and PRC or PRA (Fig. 4a, b). These results suggested that serum s(P)RR and that the aliskiren-induced reduction of PAC were also independent of circulatory RAAS.

Serum s(P)RR level is thought to be a biomarker for the activity of the tissue RAAS. Nguyen et al. [19] have shown that plasma s(P)RR concentrations are independent of renin,

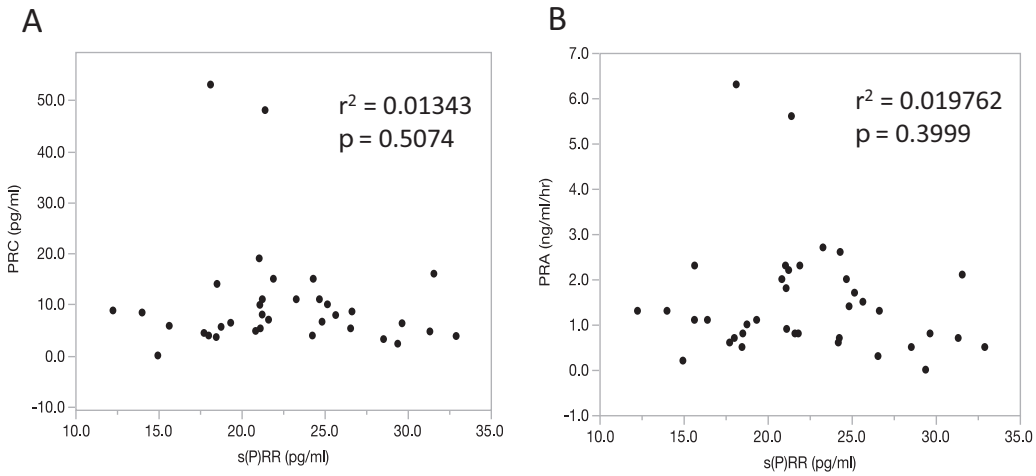


**Fig. 4** Scatter diagrams of baseline serum soluble (pro)renin receptor [s(P)RR] and plasma renin concentration (PRC) (**a**) and plasma renin activity (PRA) (**b**)

prorenin, and aldosterone concentrations in healthy subjects and in patients with contrasted degrees of circulatory RAAS activity. However, as for the relation between tissue RAAS activity and s(P)RR, we have previously shown that serum s(P)RR level was associated with CKD (decreased eGFR) [1]. (P)RR expression increased in the remnant kidney of 5/6 nephrectomized rats, a model of CKD [20]. We have also reported that serum s(P)RR level was positively correlated with urinary angiotensinogen [1], a biomarker for intrarenal RAAS [21]; the level is also reported to be higher in HD patients [22], who are believed to have increased intrarenal RAAS activity [23, 24]. Tissue RAAS activity is also thought to be increased in patients with diabetes mellitus. It has been reported that in pregnant women, increased level of s(P)RR in the first trimester may be a marker for predicting gestational diabetes mellitus [25, 26]. This may also supports the concept that serum s(P)RR level may reflect tissue (P)RR expression, i.e., tissue RAAS activity, but further investigation is needed for the direct evidence to proof this mechanism.

How AngII/AngII type 1 receptor induced aldosterone synthesis in human adrenal gland is regulated remains unclear [27]. Accumulated evidence has shown that local secretory RAAS exists in the adrenal cortex, in addition to circulating RAAS, and stimulates the production of aldosterone. (P)RR is expressed in the normal adrenal gland, both in the adrenal cortex and the medulla. It is expressed dominantly in the zona glomerulosa and the zona reticulatris [28]. Moreover, Recarti et al. [29] recently showed that (P)RR mRNA was translated to a functional protein, particularly in the sub-capsular zona glomerulosa, which is the main site of aldosterone synthesis. It has also been reported that (P)RR was overexpressed in the tumor tissue of aldosterone-producing adenomas [28]. HAC15 adrenocortical carcinoma cells incubated with either prorenin or renin exhibited a marked increase in the expression of aldosterone synthase. As aliskiren and irbesartan both inhibited this increase, it was clear that aldosterone production was strictly dependent on AngII generation and its activation of AngII type 1 receptor [29]. In transgenic rats overexpressing the human (P)RR gene, elevation in SBP was observed, in addition to an increase in PAC in the absence of changes in PRA. This suggested that (P)RR overexpression resulted in increased intraadrenal Ang II, consequently inducing an increase in aldosterone production, independent of PRA [30]. These results suggested that local secretory RAAS exists in the adrenal cortex and (P)RR may play an important role in the production of aldosterone. As a biomarker of tissue RAAS activity, serum s(P)RR may reflect aldosterone production via (P)RR in the adrenal gland.

Aliskiren suppressed aldosterone production in patients with a high level of serum s(P)RR, who may have exaggerated local secretory RAAS activity in the adrenal cortex. Hence, indicates that it inhibits aldosterone production through mechanisms associated with (P)RR. The (P)RR is capable of binding both prorenin and renin. The binding of renin causes a 4–5 fold increase in catalytic activity and the binding of prorenin confers an enzymatic activity comparable with that of renin. Aliskiren is known to bind and block the catalytic function of renin and inhibits the enhancement of renin activity by receptor-bound renin and prorenin, which therefore prevents the excess generation of AngII in tissues. As the PAC reduction observed in the DRI group appeared to be independent of circulatory RAAS, aliskiren may have decreased aldosterone synthesis predominantly through the blocking of the catalytic sites of (P)RR bound prorenin/renin, i.e., tissue RAAS, rather than circulating free renin.

There were several limitations to this study. One limitation was that the trial population was relatively small in number. Another weakness was that the treatment period was too short to examine hard outcomes. In this study, aliskiren appeared to exert beneficial effects on patients with high basal serum s(P)RR level regarding reduction in PAC, but this tendency was not observed in the changes in other parameters, such as UAE, UPE, and CAVI. A longer observation period may be needed to fully evaluate these indices.

In conclusion, we have shown that has the renoprotective effect and beneficial effect on arterial stiffness following single-RAAS blockade with aliskiren compared with a single treatment of amlodipine. To our knowledge, this is the first report of a clinical link between serum s(P)RR level and PAC response to aliskiren treatment. We have also demonstrated that s(P)RR level and aldosterone reduction were independent of circulatory RAAS, which may lead us to an idea that they may be affected more by the activity of tissue RAAS, instead. Aldosterone reduction by aliskiren may have led to the favorable outcomes for renal and vascular functions, but in the present study, we were unable to confirm this speculation. Further examination is needed to clarify the role of (P)RR, i.e., tissue RAAS, on aldosterone production and the significance of serum s(P)RR level measurements.

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## Compliance with ethical standards

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

## References

1. Morimoto S, Ando T, Niiyama M, Seki Y, Yoshida N, Watanabe D, Kawakami-Mori F, Kobori H, Nishiyama A, Ichihara A. Serum soluble (pro)renin receptor levels in patients with essential hypertension. Hypertens Res: Off J Jpn Soc Hypertens. 2014;37:642–8.
2. Watanabe N, Bokuda K, Fujiwara T, Suzuki T, Mito A, Morimoto S, Jwa SC, Egawa M, Arai Y, Suzuki F, Sago H, Ichihara A. Soluble (pro)renin receptor and blood pressure during pregnancy: a prospective cohort study. Hypertension. 2012;60:1250–6.
3. Hirose T, Hashimoto M, Totsune K, Metoki H, Asayama K, Kikuya M, Sugimoto K, Katsuya T, Ohkubo T, Hashimoto J, Rakugi H, Takahashi K, Imai Y. Association of (pro)renin receptor gene polymorphism with blood pressure in Japanese men: the Ohasama study. Am J Hypertens. 2009;22:294–9.
4. Feldman DL, Jin L, Xuan H, Contrepas A, Zhou Y, Webb RL, Mueller DN, Feldt S, Cumin F, Maniara W, Persohn E, Schuetz H, Jan Danser AH, Nguyen G. Effects of aliskiren on blood pressure, albuminuria, and (pro)renin receptor expression in diabetic TG (mRen-2)27 rats. Hypertension. 2008;52:130–6.
5. McMurray JJ, Pitt B, Latini R, Maggioni AP, Solomon SD, Keefe DL, Ford J, Verma A, Lewsey J. Aliskiren Observation of Heart Failure Treatment I. Effects of the oral direct renin inhibitor aliskiren in patients with symptomatic heart failure. Circ Heart Fail. 2008;1:17–24.
6. Gheorghiade M, Bohm M, Greene SJ, Fonarow GC, Lewis EF, Zannad F, Solomon SD, Baschiera F, Botha J, Hua TA, Gimpelewicz CR, Jaumont X, Lesogor A, Maggioni AP, Investigators A. Coordinators. Effect of aliskiren on postdischarge mortality and heart failure readmissions among patients hospitalized for heart failure: the ASTRONAUT randomized trial. JAMA. 2013;309:1125–35.
7. McMurray JJ, Abraham WT, Dickstein K, Kober L, Massie BM, Krum H. Aliskiren, ALTITUDE, and the implications for ATMOSPHERE. Eur J Heart Fail. 2012;14:341–3.
8. Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N, Eknoyan G. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int. 2005;67:2089–2100.
9. Saji N, Kimura K, Shimizu H, Kita Y. Silent brain infarct is independently associated with arterial stiffness indicated by cardio-ankle vascular index (CAVI). Hypertens Res: Off J Jpn Soc Hypertens. 2012;35:756–60.
10. Takase H, Dohi Y, Kimura G. Distribution of central blood pressure values estimated by Omron HEM-9000AI in the Japanese general population. Hypertens Res: Off J Jpn Soc Hypertens. 2013;36:50–57.
11. Fujime M, Tomimatsu T, Okaue Y, Koyama S, Kanagawa T, Taniguchi T, Kimura T. Central aortic blood pressure and augmentation index during normal pregnancy. Hypertens Res: Off J Jpn Soc Hypertens. 2012;35:633–8.
12. Watanabe K, Mori T, Iwasaki A, Kimura C, Matsushita H, Shinohara K, Wakatsuki A. Increased oxygen free radical production during pregnancy may impair vascular reactivity in preeclamptic women. Hypertens Res: Off J Jpn Soc Hypertens. 2013;36:356–60.
13. Morimoto S, Yurugi T, Aota Y, Sakuma T, Jo F, Nishikawa M, Iwasaka T, Maki K. Prognostic significance of ankle-brachial index, brachial-ankle pulse wave velocity, flow-mediated dilation, and nitroglycerin-mediated dilation in end-stage renal disease. Am J Nephrol. 2009;30:55–63.
14. Parving HH, Persson F, Lewis JB, Lewis EJ, Hollenberg NK, Investigators AS. Aliskiren combined with losartan in type 2 diabetes and nephropathy. N Engl J Med. 2008;358:2433–46.
15. Abe M, Maruyama N, Suzuki H, Fujii Y, Ito M, Yoshida Y, Okada K, Soma M. Additive renoprotective effects of aliskiren on angiotensin receptor blocker and calcium channel blocker treatments for type 2 diabetic patients with albuminuria. Hypertens Res: Off J Jpn Soc Hypertens. 2012;35:874–81.
16. Otsuka K, Fukuda S, Shimada K, Suzuki K, Nakanishi K, Yoshiyama M, Yoshikawa J. Serial assessment of arterial stiffness by cardio-ankle vascular index for prediction of future cardiovascular events in patients with coronary artery disease. Hypertens Res: Off J Jpn Soc Hypertens. 2014;37:1014–20.
17. Schillaci G, Battista F, Settimi L, Anastasio F, Pucci G. Cardio-ankle vascular index and subclinical heart disease. Hypertens Res: Off J Jpn Soc Hypertens. 2015;38:68–73.
18. Raptis AE, Markakis KP, Mazioti MC, Ikonomidis I, Maratou EP, Vlahakos DV, Kotsifaki EE, Voumvourakis AN, Tsirogianni AG, Lambadiari VA, Lekakis JP, Raptis SA, Dimitriadis GD. Effect of aliskiren on circulating endothelial progenitor cells and vascular

function in patients with type 2 diabetes and essential hypertension. Am J Hypertens. 2015;28:22–29.
19. Nguyen G, Blanchard A, Curis E, Bergerot D, Chambon Y, Hirose T, Caumont-Prim A, Tabard SB, Baron S, Frank M, Totsune K, Azizi M. Plasma soluble (pro)renin receptor is independent of plasma renin, prorenin, and aldosterone concentrations but is affected by ethnicity. Hypertension. 2014;63:297–302.
20. Hirose T, Mori N, Totsune K, Morimoto R, Maejima T, Kawamura T, Metoki H, Asayama K, Kikuya M, Ohkubo T, Kohzuki M, Takahashi K, Imai Y. Increased expression of (pro)renin receptor in the remnant kidneys of 5/6 nephrectomized rats. Regul Pept. 2010;159:93–99.
21. Kobori H, Alper AB Jr., Shenava R, Katsurada A, Saito T, Ohashi N, Urushihara M, Miyata K, Satou R, Hamm LL, Navar LG. Urinary angiotensinogen as a novel biomarker of the intrarenal renin-angiotensin system status in hypertensive patients. Hypertension. 2009;53:344–50.
22. Amari Y, Morimoto S, Nakajima F, Ando T, Ichihara A. Serum Soluble (Pro)Renin Receptor Levels in Maintenance Hemodialysis Patients. PLoS ONE. 2016;11:e0158068.
23. Ohashi N, Isobe S, Ishigaki S, Suzuki T, Ono M, Fujikura T, Tsuji T, Kato A, Ozono S, Yasuda H. Intrarenal renin-angiotensin system activity is augmented after initiation of dialysis. Hypertens Res: Off J Jpn Soc Hypertens. 2017;40:364–70.
24. Urushihara M, Kobori H. Intrarenal renin-angiotensin system activation in end-stage renal disease. Hypertens Res: Off J Jpn Soc Hypertens. 2017;40:351–2.
25. Bonakdaran S, Azami G, Tara F, Poorali L. Soluble (Pro) Renin Receptor is a predictor of gestational diabetes mellitus. Curr Diabetes Rev. 2016;13:555–9.
26. Watanabe N, Morimoto S, Fujiwara T, Suzuki T, Taniguchi K, Mori F, Ando T, Watanabe D, Kimura T, Sago H, Ichihara A. Prediction of gestational diabetes mellitus by soluble (pro)renin receptor during the first trimester. J Clin Endocrinol Metab. 2013;98:2528–35.
27. Miura SI, Suematsu Y, Matsuo Y, Tomita S, Nakayama A, Goto M, Arimura T, Kuwano T, Yahiro E, Saku K. The angiotensin II type 1 receptor-neprilysin inhibitor LCZ696 blocked aldosterone synthesis in a human adrenocortical cell line. Hypertens Res: Off J Jpn Soc Hypertens. 2016;39:758–63.
28. Yamamoto H, Kaneko K, Ohba K, Morimoto R, Hirose T, Satoh F, Totsune K, Takahashi K. Increased expression of (pro)renin receptor in aldosterone-producing adenomas. Peptides. 2013;49:68–73.
29. Recarti C, Seccia TM, Caroccia B, Gonzales-Campos A, Ceolotto G, Lenzini L, Petrelli L, Belloni AS, Rainey WE, Nussberger J, Rossi GP. Expression and functional role of the prorenin receptor in the human adrenocortical zona glomerulosa and in primary aldosteronism. J Hypertens. 2015;33:1014–22.
30. Burckle CA, Jan Danser AH, Muller DN, Garrelds IM, Gasc JM, Popova E, Plehm R, Peters J, Bader M, Nguyen G. Elevated blood pressure and heart rate in human renin receptor transgenic rats. Hypertension. 2006;47:552–6.