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ORIGINAL ARTICLE

Additive renoprotective effects of aliskiren on angiotensin receptor blocker and calcium channel blocker treatments for type 2 diabetic patients with albuminuria

Masanori Abe[1], Noriaki Maruyama[1], Hiroko Suzuki[1], Yuki Fujii[1], Midori Ito[1], Yoshinori Yoshida[1], Kazuyoshi Okada[1] and Masayoshi Soma[1,2]

This open-label, randomized, parallel-controlled study investigated the effects of the direct renin inhibitor aliskiren on 64 hypertensive type 2 diabetic patients with chronic kidney disease (CKD) and stable glycemic control who were already being treated with fixed doses of antihypertensive agents over a 24-week period. These agents were 80 mg of the angiotensin II receptor blocker (ARB) telmisartan and 5 mg of the calcium channel blocker (CCB) amlodipine. Patients were randomly assigned to two groups: the aliskiren group, receiving 150 mg per day aliskiren, which was increased to 300 mg per day ($n=32$), and the CCB group, which received an increased dose (7.5 mg per day) of amlodipine that was increased to 10 mg per day ($n=32$). Urinary albumin excretion and urinary levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) and liver-type fatty acid-binding protein (L-FABP) were investigated in each group. Mean systolic and diastolic blood pressure decreased significantly in both groups, but there was no significant difference between the two groups at the end of the study. Serum creatinine levels and estimated glomerular filtration rate did not differ significantly between the two groups, but percent changes of urinary albumin/creatinine ratios, 8-OHdG and L-FABP levels decreased significantly in the aliskiren group compared with the CCB group. Plasma aldosterone levels were significantly decreased in the aliskiren group, which correlated significantly with those of urinary 8-OHdG and L-FABP. Our results suggest that the addition of aliskiren to the maximal recommended dose of ARB and usual dose of amlodipine is more effective in reducing albuminuria and oxidant stress in hypertensive diabetic patients with CKD than increasing the dose of amlodipine.
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Keywords: aldosterone; aliskiren; chronic kidney disease; diabetic nephropathy; oxidant stress

INTRODUCTION

The importance of strict blood pressure (BP) control in patients with chronic kidney disease (CKD) was emphasized by the Japanese Society of Hypertension, which recommends a target BP of $<130/80$ mm Hg for hypertensive patients with CKD.[1] However, despite the high prevalence of hypertension in patients with CKD (81.8%), only 65.9% receive antihypertensive therapy, and in those that do receive treatment, only 23.3% are able to control their BP.[2]

Reduction in proteinuria using suitable therapeutic interventions, such as the antihypertensive regimen renin–angiotensin–aldosterone system (RAAS) blockade, is associated with a slower decline in renal function compared with other forms of treatment, yet RAAS blockade with angiotensin-converting enzyme inhibitors or angiotensin II type-1 receptor blockers (ARBs) is currently considered the most effective pharmacological approach for renoprotection.[3,4] These agents reduce proteinuria more effectively than other antihypertensive drugs, therefore RAAS inhibitors should be titrated to maximal recommended doses.[1,5] However, it is difficult to control BP with monotherapy, especially in patients with CKD, thus highlighting the need for combination drug therapy.[6] In Japan, adequate BP control is achieved in $<50\%$ patients, and in even fewer with co-morbidities such as diabetes mellitus.[7]

The RAAS, a major regulator of BP, is an important therapeutic target for antihypertensive therapy.[8] Although angiotensin-converting enzyme inhibitors and ARBs are effective for both controlling BP and reducing proteinuria, these antihypertensives do not completely suppress the RAAS, leading to a reactive rise in plasma renin activity. The renin and (pro) renin receptor system has also been

[1]Division of Nephrology, Hypertension and Endocrinology, Department of Internal Medicine, Nihon University School of Medicine, Tokyo, Japan and [2]Division of General Medicine, Department of Internal Medicine, Nihon University School of Medicine, Tokyo, Japan
Correspondence: Dr M Abe, Division of Nephrology, Hypertension and Endocrinology, Department of Internal Medicine, Nihon University School of Medicine, 30-1, Oyaguchi Kami-chou, Itabashi-ku, Tokyo 173-8610, Japan.
E-mail: abe.masanori@nihon-u.ac.jp
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shown to have a role in the development and progression of CKD.[9,10] As angiotensin-converting enzyme inhibitors and/or ARBs increase prorenin levels and plasma renin activity, inhibition of renin is a potential therapeutic target for CKD patients, especially those who have received RAAS inhibitors.[9,10]

Aliskiren is a newly developed, orally active direct renin inhibitor for the treatment of hypertension,[11–14] which has been shown by Parving *et al.*[15,16] to reduce albuminuria in hypertensive diabetic patients with nephropathy who had already been treated with the maximal recommended dose of ARB (losartan 100 mg daily). These findings further suggest the clinical utility of the dual blockade of RAAS with aliskiren and ARB for the treatment of CKD. Although it has been reported that the addition of aliskiren to olmesartan, another ARB, decreases not only urinary protein excretion but also urinary liver-type fatty acid-binding protein (L-FABP) levels in non-diabetic CKD stage 1–2 patients,[17] the relationship between changes in these urinary markers and changes in plasma aldosterone and plasma renin activity levels are not well known, particularly in diabetic subjects with CKD. Therefore, the objective of this study was to determine the effects of additional aliskiren therapy or dose titration therapy of amlodipine in hypertensive diabetic patients with CKD, who were already being treated with the maximal recommended dose of ARB (80 mg telmisartan) and the usual dose of calcium channel blocker (CCB; 5 mg amlodipine), on BP control and renoprotection.

METHODS

Subjects and study design

Sixty-four patients were enrolled in the study. Enrollment criteria were as follows: (1) treatment with a daily ARB dose of 80 mg telmisartan and a daily CCB dose of 5 mg amlodipine for at least 8 weeks before the study; (2) type 2 diabetes with nephropathy under stable glycemic control, defined as a hemoglobin A1c (HbA1c) level $<7.0\%$ after administration of oral anti-diabetic agents or insulin injection therapy for 8 consecutive weeks; (3) albuminuria: urinary albumin/creatinine (Cr) ratio $>30\,mg\,g^{-1}$ (average of two consecutive measurements recorded during the 8-week pre-treatment period); (4) estimated glomerular filtration rate (eGFR) levels 30–89 $ml\,min^{-1}$ per $1.73\,m^2$; (5) hypertension: systolic and diastolic BP $\geqslant$130/80 mm Hg recorded while the patient was in a sitting position during at least two independent clinic visits.

Exclusion criteria were as follows: (1) age <20 years or >80 years; (2) more than second-degree hypertension (BP $>$160/100 mm Hg)[1]; (3) massive albuminuria: urinary albumin/Cr ratio $>2000\,mg\,g^{-1}$ (average of two consecutive measurements recorded during the 8-week pre-treatment period); (4) eGFR $\geqslant 90\,ml\,min^{-1}$ per $1.73\,m^2$ or $<30\,ml\,min^{-1}$ per $1.73\,m^2$; (5) severe heart failure, angina, myocardial infarction or stroke occurring within 6 months from the start of the trial; and (6) diabetes with unstable glycemic control, defined as a HbA1c level of $\geqslant 7.0\%$ after administration of anti-diabetic agents for 8 consecutive weeks.

An overview of the study design is shown in Figure 1. An independent investigator, who did not treat and had no knowledge of the subjects, monitored randomized subject entry, and subjects were randomly split into two groups; assignment particulars were then immediately delivered to individual investigators. The randomization method was modified by dynamic balancing of the serum creatinine (sCr), eGFR and urinary albumin/Cr ratio values recorded at the time of patient registration.

After initial evaluation, the aliskiren group ($n=32$) received 150 mg per day aliskiren, which was increased up to 300 mg per day, while the CCB group ($n=32$) received an additional 2.5 mg per day amlodipine (giving a total daily dose of 7.5 mg amlodipine), which was increased to 5 mg per day (total daily dose of 10 mg amlodipine) over a 24-week period.

The study was conducted with outpatients at our institutions who fulfilled the criteria. The study protocol was approved by the local ethics committee and all patients gave their written informed consent for participation. The study was conducted in accordance with the Declaration of Helsinki.

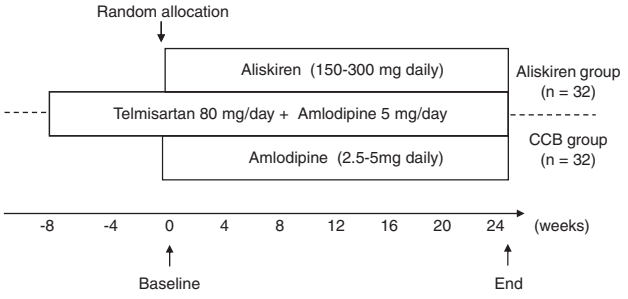

Figure 1 Study design.

Intervention

Doses of other anti-hypertensive agents and statins were maintained during the study. BP measurements were performed at the outpatient clinic according to the Japanese Society of Hypertension 2009 guidelines at fixed times after the administration of medications.[1] The target BP was $<$130/80 mm Hg. Measurements were made using a sphygmomanometer (Nippon Colin, Tokyo, Japan) and were performed twice with the patient in a sitting position after a 5-min rest. Patients were given dietary guidance, especially those under dietary restrictions.

Biochemical and urinary assessments

At each patient visit, safety variables and patient compliance with treatment regimens were assessed. Laboratory tests, including those for measuring sCr, fasting plasma glucose, HbA1c, hemoglobin (Hb), aspartate aminotransferase, alanine aminotransferase, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, sodium, potassium and uric acid levels, were performed using commercial kits employing routinely used clinical chemistry procedures. High-sensitivity C-reactive protein was measured by latex agglutination.

Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) and L-FABP were measured at baseline before treatment and at week 24 by a specific enzyme linked immunosorbent assay (SRL, Tokyo, Japan) in the first morning urine sample, and values were expressed as a ratio to the urinary Cr concentration. Urinary albumin excretion was assessed by measuring urinary concentrations of albumin and Cr (albumin/Cr ratio) in the first morning urine sample. Urinary albumins were measured using the immunoturbidimetric assay. Plasma renin activity and aldosterone concentrations were measured by radioimmunoassay at a contract laboratory (SRL) at baseline and at the end of the study in the supine position after a 20-min rest. The eGFR was calculated using the following formula:[18] eGFR ($ml\,min^{-1}$ per $1.73\,m^2$) $= 194 \times sCr^{-1.094} \times age^{-0.287}$ ($\times 0.739$ for women).

Statistical analysis

All values are expressed as mean $\pm$ s.e.m. We used the unpaired *t*-test or Fisher's exact test to compare baseline characteristics of patients in the aliskiren and CCB groups. Differences between the two groups with regard to changes in systolic and diastolic BPs were evaluated by repeated-measures analysis of variance according to a general linear model. If the analysis of variance revealed a significant overall difference, *a priori*-defined pairwise comparisons were performed using the Student's *t*-test without adjustment for multiple tests. Intragroup changes in sCr values and the urinary albumin/Cr ratio were analyzed using the paired *t*-test. The unpaired *t*-test was used to compare sCr values and the urinary albumin/Cr ratio between the aliskiren and CCB groups. Correlations were determined by the Spearman's rank correlation test. To identify independent determinants of albuminuria, urinary 8-OHdG and L-FABP, multiple stepwise regression analysis was performed. Statistical significance was set at $P<0.05$.

RESULTS

Baseline characteristics

All enrolled patients ($n=64$) remained in the study until the end of the trial. Baseline characteristics and initial medication are shown in Table 1. There were no significant differences between the two groups with regard to baseline characteristics.

BP-lowering effect

The final dose of aliskiren was 206 ± 13 mg per day in the aliskiren group, and that of amlodipine was 3.8 ± 0.3 mg per day (total daily dose, 8.8 ± 0.3 mg) in the CCB group. Figure 2 shows the changes in systolic and diastolic BP. In both groups, systolic and diastolic BP values were significantly lower than baseline values after the initiation of additional therapy, but the values did not differ significantly between the two groups during the course of treatment. In the last month of treatment, no significant differences (NS) were observed between systolic BP (aliskiren group: 128 ± 2 mm Hg, CCB group: 129 ± 1 mm Hg) and diastolic BP (aliskiren group: 74 ± 2 mm Hg, CCB group: 74 ± 2 mm Hg) in the two groups.

The BP target value (130/80 mm Hg) was achieved in 56 and 59% of all subjects in the aliskiren and CCB groups (NS), respectively. The heart rate showed no significant change between baseline and the end of the study in both groups, and there was no significant difference between the two groups at the end of the trial.

Plasma renin activity and aldosterone levels

As shown in Figure 3, plasma renin activity was significantly decreased in the aliskiren group after 24 weeks of treatment, and the difference at the end of the study between the two groups was significant (aliskiren group: 0.3 ± 0.1 ng ml^{-1} per h, CCB group: 2.7 ± 0.5 ng ml^{-1} per h, $P<0.05$). Plasma aldosterone levels were also significantly decreased in the aliskiren group after 24 weeks of treatment, and the difference at the end of the study between the two groups was significant (aliskiren group: 68 ± 6 pg ml^{-1}, CCB group: 101 ± 8 pg ml^{-1}, $P<0.01$). However, no significant effects were observed in the CCB group.

Anti-albuminuric effects

Figure 4 shows that neither sCr levels nor eGFR levels differed significantly between the two groups at the end of the study (aliskiren group sCr: 1.2 ± 0.1 mg dl^{-1}, CCB group sCr: 1.1 ± 0.1 mg dl^{-1}, NS; aliskiren group eGFR: 47 ± 2 ml min^{-1} per 1.73 m^2, CCB group eGFR: 48 ± 2 ml min^{-1} per 1.73 m^2, NS).

As shown in Figure 5, the urinary albumin/Cr ratio was significantly decreased in the aliskiren group (from 577 ± 88 to 352 ± 57 mg g^{-1} Cr, $P<0.0001$), but there was no significant change in the urinary albumin/Cr ratio in the CCB group. Furthermore, the percent changes from the baseline value of the urinary albumin/Cr ratio recorded for the aliskiren group were significantly lower compared with the CCB group (aliskiren group: -40 ± 4%, CCB group: -0.4 ± 15%, $P<0.01$).

Urinary 8-OHdG and L-FABP

Urinary 8-OHdG levels significantly decreased from 15.5 ± 1.8 ng mg^{-1} Cr to 11.5 ± 0.8 ng mg^{-1} Cr after 24 weeks in the aliskiren group ($P<0.01$; Figure 6). Furthermore, there were significant differences in urinary 8-OHdG levels between the two groups at the end of the study (aliskiren group: 11.5 ± 0.8 ng mg^{-1} Cr; CCB group: 14.6 ± 1.2 ng mg^{-1} Cr, $P<0.05$). Urinary L-FABP levels also significantly decreased from 20.7 ± 3.0 ng mg^{-1} Cr to 12.5 ± 1.9 ng mg^{-1} Cr after 24 weeks in the aliskiren group ($P<0.001$), and there were significant differences in these levels between the two groups at the end of the study (aliskiren group: 12.5 ± 1.9 ng mg^{-1} Cr; amlodipine group: 19.9 ± 2.8 ng mg^{-1} Cr, $P<0.05$).

On the other hand, the CCB group showed little difference in urinary levels of 8-OHdG and L-FABP during the study period (Figure 6). The percent changes from the baseline value of urinary 8-OHdG and L-FABP recorded for the aliskiren group were significantly lower than those of the CCB group (both $P<0.001$).

Assessment of the factors related to albuminuria, urinary 8-OHdG and L-FABP levels

As shown in Table 2, univariate analyses were performed to examine the relationships between changes in albuminuria, urinary 8-OHdG and L-FABP levels and other variables in the aliskiren group. The change in albuminuria correlated with changes in fasting plasma glucose, HbA1c and low-density lipoprotein cholesterol. Multiple stepwise regression analysis found that only HbA1c was independently related to albuminuria. The change in urinary 8-OHdG correlated with changes in urinary L-FABP, high-density lipoprotein cholesterol and plasma aldosterone. Multivariate regression analysis showed that urinary L-FABP was a significant independent variable associated with changes in urinary 8-OHdG. Moreover, the change in

Table 1 Patient baseline characteristics and medications

Variables	*Aliskiren group*	*CCB group*	P-*value*
N (male/female)	32 (17/15)	32 (18/14)	NS
Age (years)	67 ± 2	68 ± 1	NS
BMI (kg m^{-2})	23.2 ± 1.7	22.9 ± 1.6	NS
Smoking (%)	28	28	NS
Dyslipidemia (%)	75	72	NS
Systolic BP (mm Hg)	144 ± 2	143 ± 1	NS
Diastolic BP (mm Hg)	83 ± 2	81 ± 2	NS
Heart rate (bpm)	74 ± 2	74 ± 2	NS
Serum Cr (mg dl^{-1})	1.2 ± 0.1	1.1 ± 0.1	NS
eGFR (ml min^{-1} per 1.73 m^2)	47 ± 2	48 ± 2	NS
Urinary albumin/Cr ratio (mg g^{-1} Cr)	577 ± 88	562 ± 95	NS
Medications (n)			
Antihypertensive agents			
Thiazide diuretic	5	4	NS
Loop diuretic	4	4	NS
α-Blocker	3	3	NS
β-Blocker	3	4	NS
ACE-inhibitor	2	1	NS
Antidiabetic agents			
Glimepiride	7	6	NS
Metformin	3	2	NS
α-GIs	4	4	NS
Pioglitazone	4	4	NS
Glinides	3	3	NS
DPP-4 inhibitors	8	10	NS
Insulin	9	8	NS
Statins	24	23	NS

Abbreviations: α-GIs, alpha-glucosidase inhibitors; ACE, angitensin-converting enzyme; BMI, body mass index; BP, blood pressure; Cr, creatinine; DPP-4, dipeptidyl peptidase-4 ; eGFR, estimated glomerular filtration rate; NS, not significant.

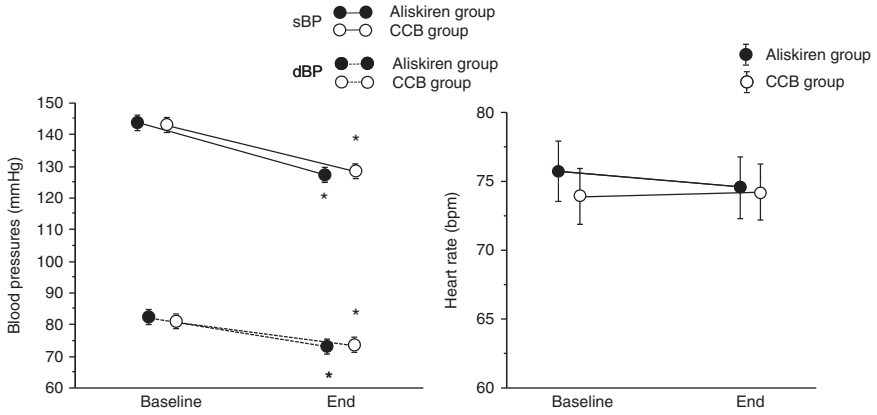

Figure 2 Changes in blood pressure and heart rate in the aliskiren group (solid circles) and calcium channel blocker (CCB) group (open circles) during the study period. $^{*}P<0.0001$ *vs.* baseline. Abbreviarions: dBP, diastolic blood pressure; sBP, systolic blood pressure.

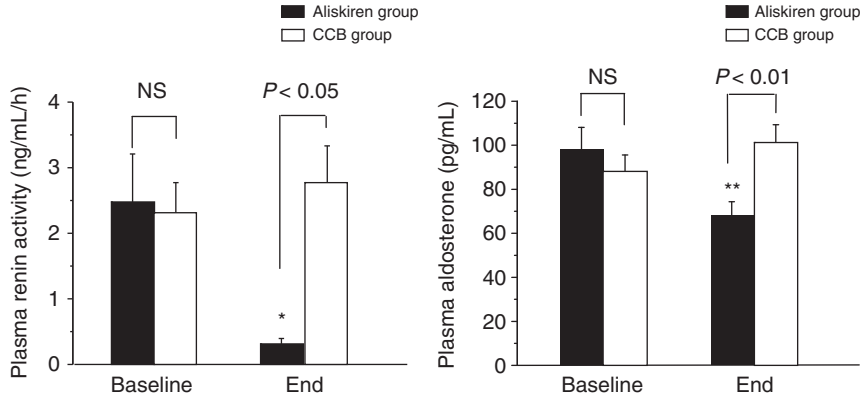

Figure 3 Changes in plasma renin activity and aldosterone levels between aliskiren group (solid bars) and calcium channel blocker (CCB) group (open bars). $^{*}P<0.01$, $^{**}P<0.001$ *vs.* baseline.

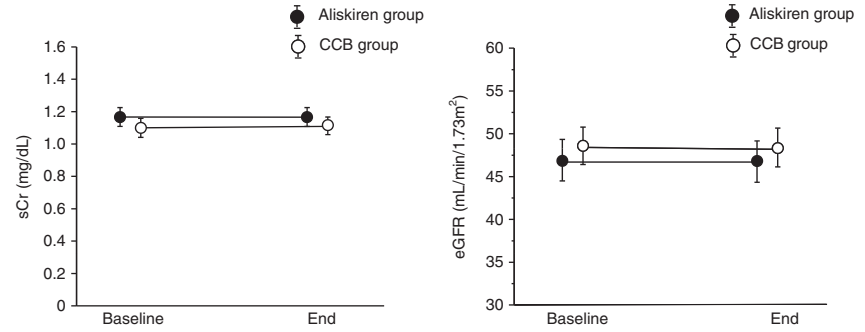

Figure 4 Changes in serum creatinine (sCr) levels and the estimated glomerular filtration rate (eGFR) between aliskiren group (solid circles) and calcium channel blocker (CCB) group (open circles).

L-FABP correlated with changes in urinary 8-OHdG, plasma renin activity and plasma aldosterone. Multivariate regression analysis also showed that urinary 8-OHdG and plasma aldosterone were significant independent variables associated with changes in urinary L-FABP.

Sub-analysis: subjects of the upper GFR and lower GFR group

Subjects in the aliskiren group were subdivided into two groups based on eGFR median levels at baseline (44.3 ml min^{-1} per 1.73 m^2): the upper GFR group (eGFR, $\geqslant$44.3 ml min^{-1} per 1.73 m^2) and the lower GFR group (eGFR, $<$44.3 ml min^{-1} per 1.73 m^2), and subgroup analysis was performed. In both the upper- and lower-GFR groups, there were no significant differences in age, dosage of aliskiren, percent changes in urinary albumin/Cr ratios, urinary 8-OHdG, L-FABP, plasma renin activity, glycemic indices or electrolytes. However, percent change of plasma aldosterone was greater in the lower GFR group compared with the upper GFR group. There was no significant difference in the change of systolic and diastolic BPs or eGFR (Table 3).

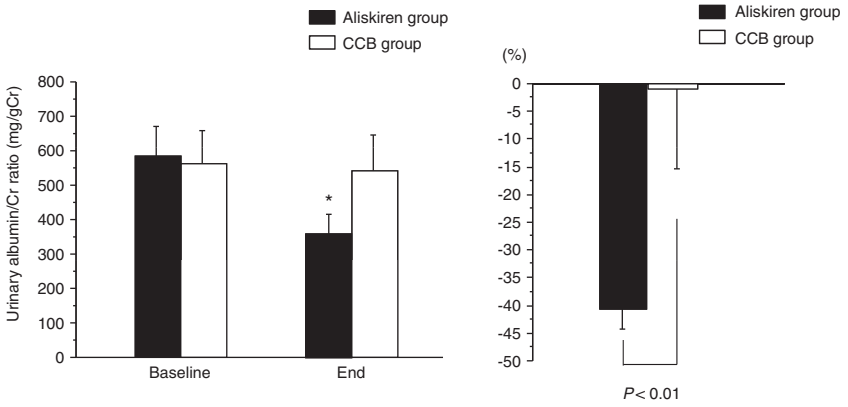

Figure 5 Changes in urinary albumin/creatinine (Cr) ratio and the corresponding percent changes from baseline between aliskiren group (solid bars) and calcium channel blocker (CCB) group (open bars). $^{*}P<0.0001$ *vs.* baseline.

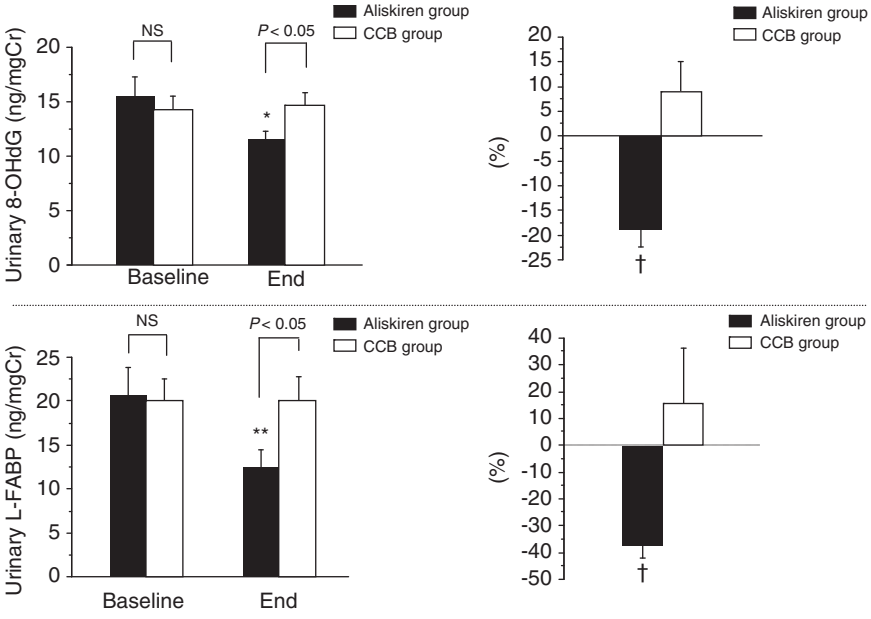

Figure 6 Changes in urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) and liver-type fatty acid-binding protein (L-FABP) levels between aliskiren group (solid bars) and calcium channel blocker (CCB) group (open bars). $^{*}P<0.01$, $^{**}P<0.001$ *vs* baseline, $^{\dagger}P<0.001$ *vs.* CCB group.

Biochemical parameters

As listed in Table 4, fasting plasma glucose levels and HbA1c levels did not differ significantly between the two groups during the study period. There were also no significant changes in total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, electrolyte or uric acid levels in either group. However, high-sensitivity C-reactive protein levels were significantly reduced in the aliskiren group.

Adverse events

During the observation period, transient increases in serum potassium levels ($>5.5\,\mathrm{mmol\,l^{-1}}$) were observed in four patients (12.5%) in the aliskiren group and in two patients (6.3%) in the CCB group, but this was not significantly different ($P=0.17$). Furthermore, three of the four patients in the aliskiren group were also in the lower GFR group. No patients in either group exhibited increases in serum potassium levels of $>6.0\,\mathrm{mmol\,l^{-1}}$. Although one patient in the aliskiren group had received calcium polystyrene sulfonate treatment, this was only short-lived, and serum potassium levels decreased to $<5.5\,\mathrm{mmol\,l^{-1}}$ after 4 weeks of treatment. Potassium levels in other patients spontaneously decreased to $<5.5\,\mathrm{mmol\,l^{-1}}$. No patients in either group exhibited a significant increase in the occurrence of adverse effects, such as hypotension, hyperglycemia, liver impairment, anemia or skin rash, and no patients required renal replacement therapy for hyperkalemia or progressive loss of renal function.

DISCUSSION

In the present study, we demonstrated that treatment with aliskiren in addition to the ARB telmisartan was more beneficial than an increase in the CCB amlodipine dose in diabetic patients with CKD. This was indicated by decreases in systolic and diastolic BP, albuminuria and urinary excretion levels of 8-OHdG and L-FABP, which are clinical biomarkers of tubulointerstitial damage.

Table 2 Univariate and stepwise multiple regression analysis for determinants of albuminuria, L-FABP and 8-OhdG in the aliskiren group

	Albuminuria				*Urinary 8-OhdG*				*Urinary L-FABP*			
	Univariate		*Multivariate*		*Univariate*		*Multivariate*		*Univariate*		*Multivariate*	
Factors	ρ	P	β	P	ρ	P	β	P	ρ	P	β	P
Albuminuria (%)					0.163	0.370	−1.172	0.153	0.133	0.465	0.239	0.138
Urinary 8-OHdG (%)	−0.066	0.708							0.535	0.0016	0.552	0.0005
Urinary L-FABP (%)	0.133	0.465	0.420	0.122	0.535	0.0016	0.547	0.0028				
BMI ($kg\,m^{-2}$)	0.170	0.349	−0.420	0.160	0.100	0.583			0.299	0.095		
Systolic BP (mm Hg)	0.211	0.244			−0.095	0.604	−0.027	0.29	0.092	0.616		
Diastolic BP (mm Hg)	0.128	0.484			0.118	0.518			0.007	0.966		
eGFR ($ml\,min^{-1}$ per $1.73\,m^2$)	−0.261	0.148			−0.069	0.705			−0.098	0.591		
Fasting plasma glucose (%)	0.356	0.045			0.161	0.377			0.033	0.857		
Hemoglobin A1c (%)	0.399	0.023	−0.239	0.010	0.062	0.734			−0.290	0.107		
LDL-cholesterol (%)	0.358	0.044	0.361	0.120	−0.126	0.489			0.250	0.167		
HDL-cholesterol (%)	0.038	0.833			0.380	0.0317	0.458	0.059	−0.121	0.509		
Triglyceride (%)	0.231	0.203			−0.057	0.756			0.146	0.424	0.035	0.054
Plasma renin activity (%)	0.018	0.921			0.046	0.802			0.351	0.048		
Aldosterone (%)	0.022	0.901			0.383	0.033	0.262	0.066	0.460	0.0081	0.392	0.0119
Hs-CRP (%)	0.140	0.443			−0.030	0.868			0.180	0.322		
			$R^2 = 0.420$				$R^2 = 0.577$				$R^2 = 0.608$	

Abbreviations: BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; Hs-CRP, high-sensitivity C-reactive protein; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; LDL, low-density lipoprotein; L-FABP, liver-type fatty acid-binding protein.

Table 3 Characteristics and changes in clinical variables according to baseline eGFR

Variables	*Upper GFR group*	*Lower GFR group*	P-*value*
Mean eGFR ($ml\,min^{-1}$ per $1.73\,m^2$)	57 ± 3	35 ± 1	
N (male/female)	17 (9/8)	15 (8/7)	NS
Age (years)	66 ± 3	71 ± 3	NS
Dosage of aliskiren (mg per day)	194 ± 17	220 ± 20	NS
Systolic BP (mm Hg)	−16 ± 2	−13 ± 2	NS
Diastolic BP (mm Hg)	−9 ± 1	−6 ± 2	NS
Urinary albumin/Cr ratio (%)	−45 ± 5	−34 ± 4	NS
Urinary L-FABP (%)	−24 ± 8	−38 ± 6	NS
Urinary 8-OHdG (%)	−15 ± 6	−18 ± 2	NS
eGFR ($ml\,min^{-1}$ per $1.73\,m^2$)	0.6 ± 0.9	0.1 ± 0.7	NS
Plasma renin activity (%)	−70 ± 6	−77 ± 4	NS
Plasma aldosterone (%)	−13 ± 8	−32 ± 6	0.035
Hs-CRP (%)	−43 ± 6	−32 ± 8	NS
Fasting plasma glucose (%)	−3 ± 2	3 ± 5	NS
Percent changes of HbA1c (%)	0.01 ± 0.04	−0.10 ± 0.05	NS
Sodium (%)	0.2 ± 0.3	0.6 ± 0.3	NS
Potassium (%)	1.4 ± 1.9	1.5 ± 3.3	NS
Uric acid (%)	−1.9 ± 1.5	−0.5 ± 3.2	NS

Abbreviations: BP, blood pressure; Cr, creatinine; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; Hs-CRP, high-sensitivity C-reactive protein; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; L-FABP, liver-type fatty acid-binding protein.

Beyond its antihypertensive effects, recent data suggest that aliskiren may have beneficial effects on renal function by slowing the decline in GFR and reducing albuminuria, which are independent of its BP-lowering effects.[15] In the AVOID study, those hypertensive patients with type 2 diabetes and nephropathy who received aliskiren and losartan combination therapy for 6 months showed a smaller decline in GFR than patients treated with placebo plus losartan, even though the BP was similar and well controlled in both treatment groups.[15] In the aliskiren plus losartan group, 25% of patients experienced a reduction in the urinary albumin/Cr ratio of >50% compared with only 12.5% patients in the placebo plus losartan group. Additional research has shown that the RAAS blockade with direct renin inhibition can also decrease albuminuria. In a study by Persson *et al.*,[19] a daily dose of 300 mg aliskiren for 28 days was associated with a 44% decrease in the urinary albumin/Cr ratio and a reduction in 24-h systolic BP (after 7 days) in patients with type 2 diabetes and albuminuria. In the present study, although the BP lowering degree of aliskiren was similar to that of amlodipine, the reduction of albuminuria in the aliskiren group was greater than in the CCB group, suggesting that aliskiren may have beneficial pleiotropic actions in diabetic patients with CKD who have already been treated with the maximal recommended dose of ARB and usual dose of CCB.

In patients with diabetic nephropathy, the development of progressive diabetic kidney disease is associated with changes in proximal tubuli, and renal function and prognosis show a higher correlation with structural lesions in the tubulointerstitium than with glomerular changes.[20] Inflammation and increased oxidative stress induced by hyperglycemia may contribute to the pathogenesis of diabetic complications including nephropathy.[21,22] When advanced glycation end-products, shear stresses and angiotensin II stimulate their respective receptors, nicotinamide adenine dinucleotide phosphate oxidase activity is enhanced and production of oxidative stress is increased.[21,23] Urinary L-FABP is an important marker of tubulointerstitial changes in diabetic nephropathy, while oxidative stress is a pathogenetic factor underlying diabetic complications such as nephropathy.[24–27]

Furthermore, diabetic hyperglycemia is associated with increased production of reactive oxygen species.[22] Reactive oxygen species damage to DNA necessitates the induction of various DNA repair

Table 4 Changes in biochemical measurements

	Aliskiren group			*CCB group*		
Variables	*Baseline*	*End*	P-*value*	*Baseline*	*End*	P-*value*
Fasting plasma glucose (mg dl^{-1})	130 ± 5	127 ± 5	0.50	123 ± 4	126 ± 4	0.27
Hemoglobin A1c (%)	6.5 ± 0.1	6.5 ± 0.1	0.30	6.4 ± 0.1	6.4 ± 0.1	0.52
Total cholesterol (mg dl^{-1})	168 ± 6	166 ± 6	0.19	177 ± 4	175 ± 4	0.72
LDL-cholesterol (mg dl^{-1})	87 ± 5	83 ± 5	0.06	86 ± 3	85 ± 3	0.40
HDL-cholesterol (mg dl^{-1})	53 ± 2	51 ± 2	0.16	57 ± 3	56 ± 3	0.29
Triglyceride (mg dl^{-1})	117 ± 7	109 ± 7	0.45	135 ± 7	129 ± 8	0.56
Sodium (mmol l^{-1})	140 ± 0.3	141 ± 0.3	0.08	141 ± 0.4	142 ± 0.4	0.07
Potassium (mmol l^{-1})	4.4 ± 0.1	4.4 ± 0.1	0.63	4.4 ± 0.1	4.4 ± 0.1	0.95
Uric acid (mg dl^{-1})	6.5 ± 0.3	6.5 ± 0.3	0.55	6.3 ± 0.2	6.4 ± 0.2	0.21
High-sensitivity CRP (mg l^{-1})	0.67 ± 0.08	0.45 ± 0.08	0.0012	0.55 ± 0.07	0.62 ± 0.10	0.24

Abbreviations: CCB, calcium channel blocker; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

processes, which can result in the urinary excretion of products such as 8-OHdG.[28] Increased urinary 8-OHdG and the risk of vascular complications may be present at early stages of diabetes.[29] For this reason, urinary 8-OHdG is a sensitive biomarker of oxidative DNA damage, and is also significantly correlated with the severity of tubulointerstitial lesions.[30] In the present study, we showed that aliskiren is more effective than amlodipine in protecting against tubulointerstitial injury and in reducing oxidative stress in diabetic patients with CKD.

Accumulating experimental and clinical evidence continues to show that excess aldosterone not only promotes the retention of sodium and body fluid but also induces cardiac and renal injury causing cardiac hypertrophy, glomerulosclerosis, renal inflammation and fibrosis.[31–33] Moreover, aldosterone induces oxidative stress in vascular cells through nicotinamide adenine dinucleotide phosphate oxidase activation, which has a central role in endothelial dysfunction and atherosclerotic vascular disease.[34–36] Therefore, targeting aldosterone synthesis and release may be clinically important in preventing cardiovascular disease. Our findings show a significant correlation between changes in serum aldosterone levels and changes in urinary 8-OHdG and L-FABP levels. Furthermore, the independent correlation between serum aldosterone and urinary L-FABP levels suggests that the aliskiren-induced decrease in urinary L-FABP is caused in part by the reduced serum aldosterone levels. Although aliskiren treatment decreased not only the urinary albumin/Cr ratio, but also the urinary 8-OHdG and/or L-FABP levels, no relationship was evident between changes in albuminuria and urinary 8-OHdG or L-FABP. Therefore, aliskiren might act independently on the glomeruli and the tubulointerstitium. Further investigations are needed to clarify whether aliskiren can indeed improve tubulointerstitial damage through the inhibition of aldosterone synthesis and subsequent oxidative stress, and whether these effects prevent the progression of renal dysfunction in diabetic patients with CKD.

The AVOID study showed that renal dysfunction, serum Cr elevation $>2.0\,\mathrm{mg\,dl^{-1}}$ and serum potassium elevations $>5.5\,\mathrm{mmol\,l^{-1}}$ were more frequent in the aliskiren group compared with the placebo group in diabetic patients with eGFR of $<60\,\mathrm{ml\,min^{-1}}$ per $1.73\,\mathrm{m^2}$.[37,38] Furthermore, the ALTITUDE study observed an increased incidence of nonfatal stroke, renal complications, hyperkalemia and hypotension in diabetic patients with CKD after 18–24 months of treatment.[39,40] In the present study, hyperkalemia was found in four patients in the aliskiren group, who were also mostly in the lower GFR group. However, the mean dosage of aliskiren in our subjects was relatively low (206 mg daily) compared with that in many other reports (300 mg daily). Therefore, the frequency of adverse events such as hyperkalemia and loss of renal function might be less in our study. Nevertheless, monitoring of renal function and electrolytes during aliskiren therapy should be considered for all patients with moderate to severe renal dysfunction.

Our study design suffered from the limitations of small sample size and short treatment duration. Moreover, changes in sCr levels were too small for adequate evaluation of the influence of aliskiren therapy. Additional long-term investigations are therefore necessary to accurately assess the renoprotective effects of aliskiren and ARB combination therapy in diabetic patients.

In conclusion, we show here that aliskiren treatment leads to a greater reduction of albuminuria, plasma aldosterone and urinary 8-OHdG and L-FABP levels than up-titration of amlodipine in diabetic patients with CKD. These aliskiren effects appear to make the drug more advantageous in terms of the progression of renal dysfunction and preventing cardiovascular tissue and organ injuries in such patients. We propose that aliskiren therapy should be considered as an additive therapeutic modality for hypertensive diabetic patients whose BP is not sufficiently controlled by RAAS-inhibition therapy with ARB or CCB and for the amelioration of tubulointerstitial injury.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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