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# Addition of aliskiren to olmesartan ameliorates tubular injury in chronic kidney disease patients partly by reducing proteinuria

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## Abstract

*Introduction*: Tubular injury is more important than glomerulopathy for renal prognosis in chronic kidney disease (CKD) patients. Numerous studies have demonstrated the active participation of the renin–angiotensin system (RAS) in CKD. However, whether addition of aliskiren, a direct renin inhibitor, to olmesartan improves renal tubular injury in CKD patients is unknown.

*Methods*: This study compared the effects of aliskiren (300 mg daily), olmesartan (40 mg daily), and its combination therapy on urinary L-fatty acid binding protein (L-FABP), a marker of tubular injury in stage I or II CKD patients. It also examined which clinical variables were independently correlated with tubular damage.

*Results*: Olmesartan or aliskiren monotherapy for 6 months comparably decreased blood pressure (BP) and proteinuria. BP and proteinuria levels were reduced more by combination therapy than by either monotherapy. Olmesartan or aliskiren decreased urinary L-FABP level, and combination therapy produced more incremental reduction in L-FABP level relative to each monotherapy. Multiple stepwise regression analysis revealed that BMI, low-density lipoprotein (LDL)-cholesterol and proteinuria were independently related to urinary L-FABP level.

*Conclusions*: The present study demonstrated that addition of aliskiren to olmesartan decreased urinary L-FABP level partly via reduction of proteinuria in stage I or II CKD patients.

## Keywords

Aliskiren, CKD, L-FABP, proteinuria, RAS

## Introduction

Proteinuria is one of the most common findings in chronic kidney disease (CKD).[1,2] Although persistent proteinuria shows the existence of glomerulopathy, it has recently been recognized that changes within tubulointerstitium are more important than glomerulopathy in terms of renal prognosis in patients with CKD.[2,3] Further, clinical data show a positive correlation of the extent of proteinuria with the severity of tubulointerstitial damage in CKD patients.[4,5]

Numerous studies have demonstrated the active participation of the renin–angiotensin system (RAS) in the pathogenesis of CKD.[6–10] Indeed, large clinical trials have demonstrated that interruption of the RAS by angiotensin-converting enzyme inhibitors (ACEIs) and/or angiotensin II type 1 receptor blockers (ARBs) could prevent the development and progression of CKD in high-risk patients such as diabetic subjects.[6–10] Further, angiotensin II, a physiologically active major substance of the RAS, not only acts as a vasopressor to cause systemic and glomerular hypertension but also works as a local hormone to elicit tubular cell apoptosis and inflammation.[11–14] These observations suggest that some beneficial effects of RAS inhibitors such as ACEIs and ARBs on CKD could be ascribed, at least in part, to their protective properties against tubulointerstitial damage.

The renin and (pro)renin receptor system has also been shown to play a role in the development and progression

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of CKD.[15–17] Since ACEIs and/or ARBs increase prorenin level and plasma renin activity,[15–17] inhibition of renin may be a therapeutic target in CKD patients, especially those who have received RAS inhibitors. Aliskiren is a newly developed, orally active direct renin inhibitor for the treatment of hypertension.[18] Recently, Parving et al. reported that aliskiren (300 mg daily) reduced albuminuria in hypertensive, diabetic patients with nephropathy who had already been treated with the maximal recommended dose of ARB (losartan 100 mg daily).[19] These findings further suggest the clinical utility of the dual blockade of the RAS with aliskiren and ARB for the treatment of CKD. However, as far as we know, whether addition of aliskiren to olmesartan, an ARB, could improve renal tubular injury in CKD patients is unknown. Therefore, in this study, we compared the effects of aliskiren (300 mg daily), maximal recommended dose of olmesartan (40 mg daily), and combination therapy on urinary L-fatty acid binding protein (L-FABP), a marker of tubular injury,[20] in stage I or II CKD patients. We also examined which anthropometric, metabolic and renal variables were independently correlated with tubular damage in our subjects.

## Subjects and methods

### Subjects

Thirty-six non-diabetic stage I or II CKD patients (21 males and 15 females) were enrolled in the present study. The enrollment criteria included (1) hypertension (BP >130/80 mmHg, measured in the sitting position on at least two separate hospital visits) or (2) stage I or II CKD (estimated glomerular filtration rate (eGFR) >60 ml/min with proteinuria). We excluded any patients with chronic pulmonary disease, collagen disease, liver disease, or neoplastic disorder, and those who had recent (<6 months) acute coronary syndrome, stroke or any acute infection. Patients younger than 20 years old or older than 70 years old, with serum creatinine (Cr) level more than 1.2 mg/dl, or with proteinuria more than 3.0 g/day were excluded. There were no patients with nephrotic syndrome. Thirty-six patients were randomly assigned into three groups: the first group was treated with 40 mg olmesartan once daily (seven males and five females, mean age 41.9 years), the second with 300 mg aliskiren once daily (seven males and five females, mean age 42.8 years), and the last with 300 mg aliskiren + 40 mg olmesartan daily (seven males and five females, mean age 40.9 years). These drugs had not previously been administered to these patients, so olmesartan and aliskiren treatments were started together. In addition, these drugs were not changed during the study period. Further, all patients were treated with salt restriction (<5 g/day NaCl), but none of them received other modulators of the RAS, such as ACEIs and anti-aldosterone agents, during the 6 months. The study protocol was approved by the local ethical committee of Shinmatsudo Central General Hospital, and informed consent was obtained from all study participants. The study complied with the principles of the Helsinki Declaration.

### Data collection

Height and weight were measured, and BMI (kilograms per meter squared) was calculated as an index of the presence or absence of obesity. BP was measured in the sitting position twice after 2 min of rest using an upright standard sphygmomanometer. Renal function was evaluated by serum Cr and blood urea nitrogen (BUN) levels, and eGFR according to the Modification of Diet in Renal Disease (MDRD) equation modified for the Japanese population.[21] Blood was drawn from the antecubital vein in the morning after a 12-h fast for determinations of lipid profiles; total cholesterol, triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C), fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA1c). These blood chemistries were measured with standard methods at Shinmatsudo Central General Hospital. Low-density lipoprotein cholesterol (LDL-C) level was calculated using Friedewald's formula. Urinary L-fatty acid binding protein (L-FABP), a marker of tubular injury, was measured with an enzyme-linked immunosorbent assay kit according to the manufacturer's protocol (CIMC, Tokyo, Japan).[20] Total urinary protein excretion levels were determined with a pyrogallol red method (Wako, Osaka, Japan).

### Statistical methods

Data were expressed as mean ± SD. To compare the parameters within each treatment group, we used the paired *t*-test. Comparisons among treatment groups were tested by ANOVA. A correlation between L-FABP and other clinical variables was determined by a linear regression analysis. To determine independent determinants of L-FABP, multiple stepwise regression analysis was performed. Statistical significance was defined as $p < 0.05$. All statistical analyses were performed with the use of the SAS system (SAS Institute, Cary, NC, USA).

## Results

Clinical variables before and after treatments are shown in Table 1. There were no significant differences of baseline data among the three groups, including anthropometric, metabolic, hemodynamic, and renal variables. Olmesartan or aliskiren monotherapy for 6 months comparably decreased systolic and diastolic BP, proteinuria, and serum Cr. Systolic and diastolic BP, proteinuria and serum Cr levels were reduced more by co-treatment of olmesartan and aliskiren; compared with each monotherapy, BP levels were significantly lower, and proteinuria and serum Cr levels had

**Table 1.** Clinical variables before and after treatments

| | Olmesartan | | Aliskiren | | Olmesartan + Aliskiren | |
|---|---|---|---|---|---|---|
| Characteristic | Before | After | Before | After | Before | After |
| Age (years) | 41.9 ± 8.3 | 42.3 ± 8.0 | 42.8 ± 7.2 | | 40.9 ± 6.4 | |
| Number (male) | 12 (7) | | 12 (7) | | 12 (7) | |
| BMI (kg/m$^2$) | 23.3 ± 1.3 | 23.5 ± 1.1 | 22.8 ± 1.3 | 22.6 ± 1.0 | 23.2 ± 1.1 | 22.8 ± 0.8 |
| Systolic BP (mmHg) | 155.8 ± 4.9 | 136.2 ± 5.0[b] | 157.6 ± 5.9 | 137.8 ± 4.0[d] | 157.3 ± 4.5 | 130.3 ± 2.8[f,g,h] |
| Diastolic BP (mmHg) | 89.5 ± 4.6 | 81.2 ± 3.5[b] | 90.2 ± 4.0 | 81.5 ± 2.3[d] | 89.3 ± 4.6 | 77.5 ± 2.7[f,g,h] |
| Serum Cr (mg/dl) | 0.71 ± 0.10 | 0.71 ± 0.10[a] | 0.74 ± 0.11 | 0.72 ± 0.10[c] | 0.72 ± 0.10 | 0.70 ± 0.10[f] |
| BUN (mg/dl) | 19.2 ± 2.5 | 18.8 ± 1.3 | 19.7 ± 2.4 | 19.2 ± 1.3 | 19.8 ± 2.6 | 19.1 ± 1.9 |
| eGFR (ml/min) | 86.5 ± 7.4 | 87.1 ± 7.4 | 82.9 ± 7.2 | 83.5 ± 6.8 | 86.0 ± 5.2 | 88.1 ± 6.2[f] |
| FPG (mg/dl) | 86.7 ± 5.5 | 87.0 ± 4.1 | 88.2 ± 5.8 | 87.8 ± 4.1 | 88.8 ± 4.6 | 86.3 ± 5.4[e] |
| HbA1c (%) | 5.5 ± 0.2 | 5.4 ± 0.1 | 5.4 ± 0.1 | 5.4 ± 0.1 | 5.4 ± 0.2 | 5.3 ± 0.2[f,g] |
| LDL-C (mg/dl) | 107.5 ± 10.7 | 106.0 ± 9.5 | 105.8 ± 11.1 | 103.8 ± 9.4[c] | 108.8 ± 10.0 | 101.7 ± 10.1[f] |
| HDL-C (mg/dl) | 63.2 ± 5.0 | 63.5 ± 5.4 | 62.7 ± 6.2 | 63.5 ± 5.7 | 64.8 ± 7.4 | 65.2 ± 7.6 |
| TG (mg/dl) | 110.7 ± 16.4 | 109.8 ± 14.9 | 111.5 ± 9.9 | 109.7 ± 8.6[c] | 109.3 ± 8.4 | 106.3 ± 8.9 |
| Proteinuria (mg/day) | 1113.3 ± 201.7 | 809.3 ± 239.2[b] | 1149.2 ± 264.9 | 833.3 ± 238.4[d] | 1163.3 ± 239.5 | 622.0 ± 355.2[f] |
| L-FABP (mg/g Cr) | 33.1 ± 10.5 | 25.6 ± 7.0[b] | 32.2 ± 12.5 | 25.5 ± 9.9[d] | 32.2 ± 12.7 | 18.2 ± 6.2[f] |

Data are shown as mean ± SD, unless otherwise indicated. [a]$p < 0.05$ vs. before olmesartan treatment, [b]$p < 0.01$ vs. before olmesartan treatment, [c]$p < 0.05$ vs. before aliskiren treatment, [d]$p < 0.01$ vs. before aliskiren treatment, [e]$p < 0.05$ vs. before aliskiren and olmesartan co-treatment, [f]$p < 0.01$ vs. before aliskiren and olmesartan co-treatment, [g]$p < 0.05$ vs. after olmesartan treatment, [h]$p < 0.05$ vs. after aliskiren treatment. BP: blood pressure, BUN: blood urea nitrogen, Cr: creatinine, eGFR: glomerular filtration rate, FPG: fasting plasma glucose, HbA1c: glycosylated hemoglobin, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, L-FABP: L-fatty acid binding protein, TG: triglycerides.

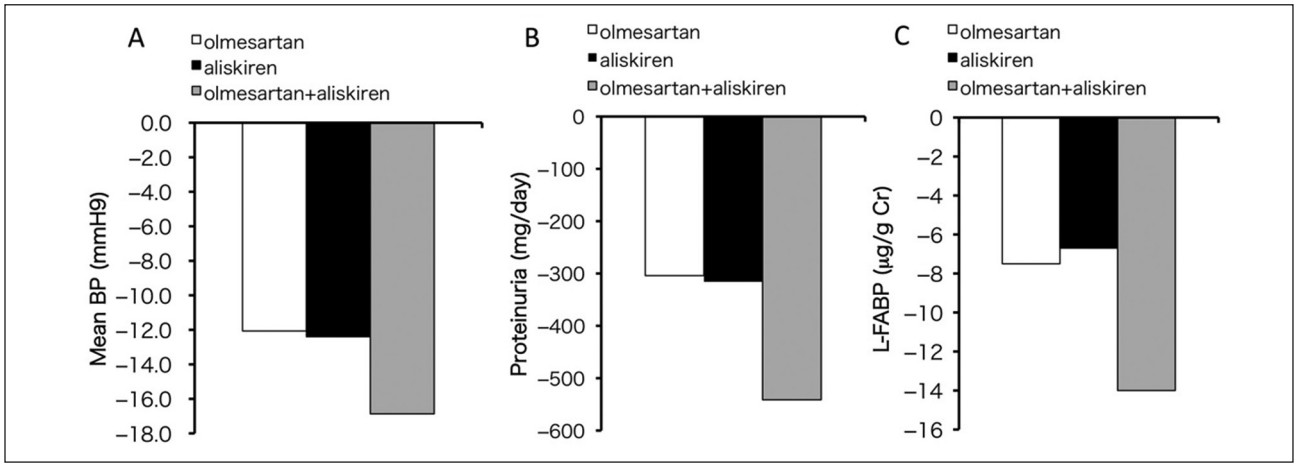


**Figure 1.** Changes from baseline in mean BP (A), urinary protein excretion (B), and urinary L-FABP (C) among each treatment group. BP: blood pressure, Cr: creatinine, L-FABP: L-fatty acid binding protein

a tendency to decrease in the olmesartan plus aliskiren group. After 6 months' treatment with olmesartan or aliskiren monotherapy, the urinary excretion level of L-FABP was reduced comparably, but the effects of each treatment on L-FABP were modest. On the other hand, olmesartan plus aliskiren treatment produced more incremental reduction in urinary L-FABP level relative to olmesartan or aliskiren therapy alone. In addition, although neither monotherapy affected FPG or HbA1c, combination therapy modestly but significantly decreased FPG and HbA1c levels. Changes from baseline in mean BP, urinary protein excretion, and urinary L-FABP are shown in

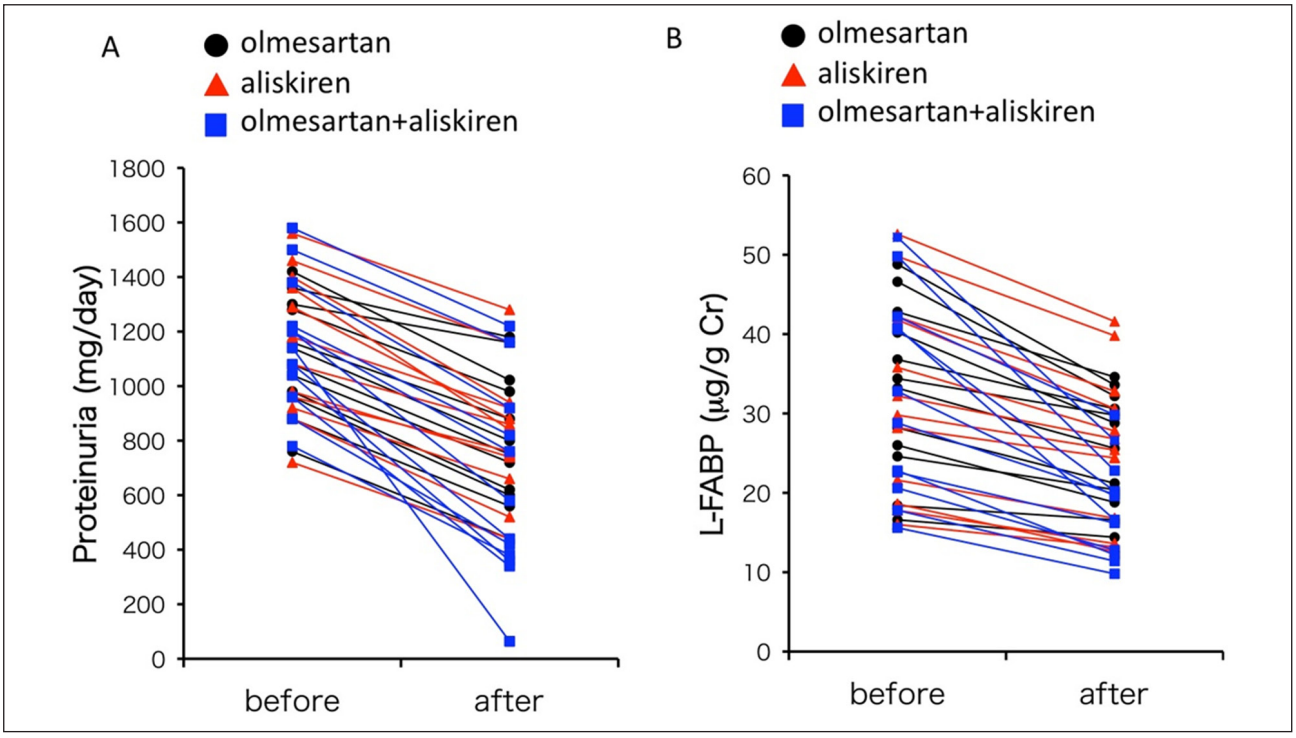


**Figure 2.** A scatterplot of the changes in urinary protein (A) and urinary L-FABP excretion (B) among each treatment group. Cr: creatinine, L-FABP: L-fatty acid binding protein

**Table 2.** Univariate and stepwise multiple regression analyses for determinants of L-FABP

| Factors | Univariate[a] | | Multivariate[b] | | |
|---|---|---|---|---|---|
| | β | *p* | β | *F* | *p* |
| BMI (kg/m²) | 0.387 | **0.01** | 0.139 | | 0.024 |
| Systolic BP (mmHg) | 0.443 | <**0.01** | | | |
| Diastolic BP (mmHg) | 0.502 | <**0.01** | | | |
| Serum Cr (mg/dl) | 0.178 | 0.135 | | | |
| BUN (mg/dl) | 0.145 | 0.226 | | | |
| eGFR (ml/min) | −0.232 | **0.050** | | | |
| FPG (mg/dl) | −0.039 | 0.743 | | | |
| HbA1c (%) | 0.323 | **0.006** | | | |
| LDL-C (mg/dl) | 0.440 | <**0.01** | 0.132 | | 0.038 |
| HDL-C (mg/dl) | 0.484 | <**0.01** | | | |
| TG (mg/dl) | 0.063 | 0.597 | | | |
| Proteinurea (mg/day) | 0.859 | <**0.01** | 0.769 | | <**0.01** |

[a]Univariate coefficients, [b]a stepwise multivariate regression analysis was performed. β: Regression coefficients. $R^2 = 0.763$. BP: blood pressure, BUN: blood urea nitrogen, Cr: creatinine, eGFR: glomerular filtration rate, FPG: fasting plasma glucose, HbA1c: glycosylated hemoglobin, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, L-FABP: L-fatty acid binding protein, TG: triglycerides.

Figure 1. A scatterplot of the changes in urinary protein and urinary L-FABP excretion is shown in Figure 2.

As shown in Table 2, univariate analyses revealed that urinary excretion level of L-FABP was correlated with BMI, systolic and diastolic BP, eGFR, HbA1c, plasma levels of LDL-C and HDL-C, and proteinuria. Because these parameters could be closely correlated with each other, to determine independent correlates of urinary L-FABP level, multiple stepwise regression analysis was performed. This analysis showed that BMI ($p < 0.05$), LDL-C ($p < 0.05$), and proteinuria ($p < 0.01$) were independently related to urinary L-FABP level ($R^2 = 0.763$) (Table 2).

## Discussion

In the present study, we demonstrated for the first time that combination therapy with aliskiren (300 mg daily) and olmesartan (40 mg daily) for 6 months decreased systolic and diastolic BP, proteinuria, and urinary excretion level of L-FABP in non-diabetic stage I or II CKD patients more than olmesartan or aliskiren monotherapy. Further, we found here that, besides BMI and LDL-C values, proteinuria was independently correlated with urinary excretion level of L-FABP, a marker of tubular injury in our subjects. Therefore, although further reductions in proteinuria with combination therapy of aliskiren and olmesartan were

modest and not significant (Table 1), our present study suggests that addition of aliskiren to olmesartan treatment may decrease urinary excretion of L-FABP level partly via reduction of proteinuria. There is accumulating evidence that proteinuria is not merely a biomarker for the progression of CKD, but also a mediator of this devastating disorder.[4,5] Indeed, albumin, one of the major components found in proteinuria, causes pro-apoptotic, pro-inflammatory and pro-fibrotic changes in cultured proximal tubular cells.[4,5,22] These observations further support the concept that tubuloprotective effects of aliskiren could be due, at least in part, to its proteinuria-lowering properties.

In this study, we did not evaluate urinary excretion levels of β2-microglobulin and/or N-acetyl-glucosaminidase, classical markers of tubular lesions.[23] However, since urinary excretion level of L-FABP is reported to accurately reflect the severity of tubulointerstitial damage,[24] our present findings suggest that co-administration of aliskiren and olmesartan is effective against tubulointerstitial injury in non-diabetic stage I or II CKD patients. Urinary L-FABP is more sensitive than urinary protein in predicting the progression of CKD.[20] Changes within tubulointerstitium are shown to be more important than glomerulopathy in terms of renal prognosis in patients with CKD.[2,3] Further, proteinuria is a strong and independent indicator of cardiovascular disease in CKD patients as well.[25] These findings suggest that co-administration of aliskiren to ARB could have beneficial effects on cardiovascular–renal systems by preventing tubular damage in non-diabetic stage I or II CKD patients partly in a BP-lowering-independent manner, that is, via reduction of proteinuria. Aliskiren (300 mg daily) added to the maximal recommended dose of ARB (losartan 100 mg daily) has been reported to provide anti-proteinuria effects, which were independent of its BP-lowering effects, in hypertensive, type 2 diabetic patients with nephropathy.[19] Aliskiren may have beneficial pleiotropic actions in CKD patients who have already been treated with the maximal recommended dose of RAS inhibitors.

In the present study, aliskiren monotherapy or combination treatment significantly decreased LDL-C level. Since LDL-C was independently correlated with urinary excretion level of L-FABP, LDL-C may play a role in tubular injury in stage I or II CKD subjects. We have previously found that treatment with statin and/or ezetimibe, a specific inhibitor of cholesterol absorption into the intestine, decreased LDL-C and urinary excretion level of L-FABP in non-diabetic stage I or II CKD patients with dyslipidemia,[26,27] thus supporting our speculation.

## Limitations

Due to relatively small numbers, this study did not have enough statistical power to draw a definite conclusion that aliskiren co-treatment with olmesartan improved tubular damage partly via reduction of proteinuria. A further large longitudinal study is needed to elucidate whether reduction of L-FABP by aliskiren co-treatment with olmesartan could be mechanistically related to cardiorenal protection in non-diabetic CKD patients.

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### Conflict of interest

There are no conflicts of intested to disclose.

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