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ORIGINAL ARTICLE

Impact of combined losartan/hydrochlorothiazide on proteinuria in patients with chronic kidney disease and hypertension

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It is unknown whether the use of diuretics is optimal over other antihypertensive agents in patients with chronic kidney disease (CKD) whose blood pressure remains uncontrolled despite treatment with renin–angiotensin system (RAS) inhibitors. In this study, we assessed the additive effects of hydrochlorothiazide (HCTZ) on reducing proteinuria in CKD patients under treatment with losartan (LS). We conducted a multicenter, open-labeled, randomized trial. One hundred and two CKD patients with hypertension and overt proteinuria were recruited from nine centers and randomly assigned to receive either LS (50 mg, $n=51$) or a combination of LS (50 mg per day) and HCTZ (12.5 mg per day) (LS/HCTZ, $n=51$). The primary outcome was a decrease in the urinary protein-to-creatinine ratio (UPCR). The target blood pressure was $<130/80$ mm Hg, and antihypertensive agents (other than RAS inhibitors and diuretics) were added if the target was not attained. Baseline characteristics of the two groups were similar. After 12 months of treatment, decreases in the UPCR were significantly greater in the LS/HCTZ group than in the LS group. There were no significant differences in blood pressure or the estimated glomerular filtration rate between the two groups. LS/HCTZ led to a greater reduction in proteinuria than treatment with LS, even though blood pressure in the LS group was similar to that in the LS/HCTZ group following the administration of additive antihypertensive agents throughout the observation period. This finding suggests that LS/HCTZ exerts renoprotective effects through a mechanism independent of blood pressure reduction.
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Keywords: angiotensin II type 1 receptor blocker; chronic kidney disease; hydrochlorothiazide

INTRODUCTION

Data from many large-scale clinical trials demonstrate that renin–angiotensin system (RAS) inhibitors such as angiotensin-converting enzyme inhibitor (ACEI) and angiotensin II type 1 receptor blocker (ARB) have an evident effect on kidney protection.[1–5] Recent guidelines on hypertension management[6,7] recommend the concomitant use of several types of antihypertensive drugs when the target blood pressure is not reached. The guidelines recommended the strict control of blood pressure in patients with chronic kidney disease (CKD) with a complication of hypertension; however, the appropriate target value of blood pressure decline is almost never reached using a single RAS inhibitor.

In various combined therapies, the combined use of RAS inhibitors such as ARB or ACEI and calcium channel blocker (CCB) or small amounts of thiazide diuretics has been determined as being effective. However, whether to select CCB or diuretics for concomitant use following RAS inhibitor is clinically an important consideration. The appropriate approach was verified by the GUARD study[8] and the ACCOMPLISH study.[9,10] Subjects of the GUARD study were diabetic nephropathy patients. Subjects of the ACCOMPLISH study were patients at high risk of cardiovascular events. Both studies compared and verified the therapeutic effects when amlodipine (a CCB) or hydrochlorothiazide (HCTZ) (a diuretic) was concomitantly used with ACEI. Although HCTZ reduced albuminuria in both studies,

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there was also a large decline in the estimated glomerular filtration rate (eGFR). In the ACCOMPLISH study, a significantly large number of cardiovascular events were generated in the HCTZ group, and the renal prognosis was also better in the CCB group. However, only a few patients exhibited overt albuminuria in both studies, and the declining rate of renal function in the patient group was also very slow.

There are no clinical studies to date comparing the effects of diuretics and the other antihypertensive agents on reducing urinary protein under treatment with ARBs and comparable blood pressure control in CKD patients with overt proteinuria. Therefore, we conducted a prospective, randomized, open-labeled, multicenter trial to determine the efficacy of a fixed-dose combination of losartan (LS) plus HCTZ and a normal dose of LS in patients with CKD and hypertension.

METHODS

The present study was a 1-year prospective, randomized, open-labeled, parallel-group, multicenter trial. The objective was to elucidate the renoprotective effects of ARB/low-dose HCTZ combination therapy on CKD patients with proteinuria and hypertension.

The protocol was approved by the Independent Review Board of Kyushu University Hospital (No. 0272) and registered at UMIN-CTR (ID: UMIN000001643). The institutional review boards or ethics committees of all participating institutions approved the study protocol. All patients provided written informed consent.

Study population

Target patients were outpatients with systolic blood pressure (SBP) >130 mm Hg and/or diastolic blood pressure (DBP) >80 mm Hg, or taking antihypertensive drugs at the time when consent was obtained. The following conditions were also required: (1) the urinary protein (mg dl^{-1})/creatinine (mg dl^{-1}) ratio (UPCR) for the 8 weeks before the study commencing exceeded 0.3 (g g^{-1} Cr); (2) eGFR was 15 ml min^{-1} per 1.73 m^2 or more; and (3) patients were aged between 20 and 74 years old. Exclusion criteria were: (1) patients with hepatic dysfunction (e.g., when alanine aminotransferase exceeded the normal upper limit by threefold or more); (2) patients who had a myocardial infarction or apoplexy in the previous 3 months; (3) patients who were or might be pregnant; (4) patients with the possibility of becoming pregnant within the study period and patients who were breastfeeding; (5) patients with a serious nephrotic syndrome (serum albumin <2 g dl^{-1}); (6) immunoglobulin A (IgA) nephropathy patients within a year from commencing steroid therapy; (7) patients with hyperkalemia (5.5 mEq l^{-1} or more); and (8) patients undergoing thiazide diuretics or thiazide-like diuretics administration.

Study design

Eligible patients were randomly assigned in a 1:1 ratio to receive either LS (50 mg per day) or LS (50 mg per day) and HCTZ (12.5 mg per day) combination therapy, each of which was administered once every morning. A 50 mg LS/12.5 mg HCTZ combination tablet was used for combination therapy. On the day of randomization, initial evaluations (medical history and medication), assessments of clinic blood pressure and laboratory tests (blood and urine) were performed after written informed consent was obtained. Figure 1 shows the study design.

ACEI or ARB administered to patients at the time of obtaining consent (-1 M) was changed to LS (50 mg per day). LS (50 mg per day) was additionally administered to patients who were not taking ACEI or ARB. LS (50 mg per day) was continued when allotted to the LS administration group at the time of commencing the allotment drug (0 M), and when allotted to the diuretic administration group, LS was replaced with an LS/HCTZ combination drug. Blood pressure measurements and blood and urine collection were carried out throughout the study period. Antihypertensive drugs other than diuretics, ACEI and ARB, were added when blood pressure did not decline to $<130/80$ mm Hg. Blood pressure measurements and blood and urine collection were carried out at 0, 1, 2, 4, 6 and 12 months following commencement of treatment.

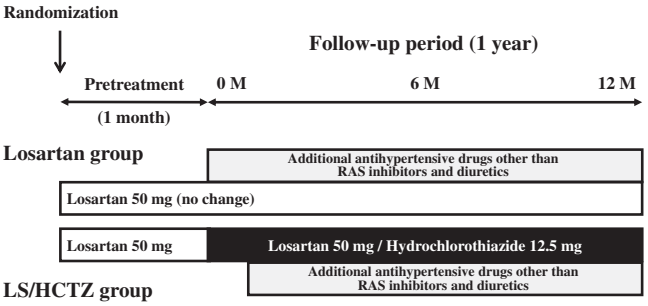

Figure 1 Study protocol. Blood pressure was targeted at $<130/80$ mm Hg during the study period. HCTZ, hydrochlorothiazide; LS, losartan; RAS, renin–angiotensin system.

Adverse events included intractable hypotension symptoms (e.g., fainting or dizziness), hyperkalemia (potassium >6.0 mEq l^{-1}), laboratory data abnormalities (e.g., acute worsening of kidney or liver function) and any side effects that required the discontinuation of medication to protect the patient's best interest.

Measurements

The levels of blood and urinary biochemical parameters and urinary protein excretion were measured in the hospital during consultations as outpatients. All assays were performed using commercially available laboratory equipment. Clinical blood pressure was measured using the auscultation method with a mercury sphygmomanometer after 5 min of rest in a seated position in the hospital. The UPCR was simultaneously estimated using casual urine samples. The eGFR was calculated according to the following formula from the Japanese Society of Nephrology: eGFR (ml min^{-1} per 1.73 m^2) $= 194 \times$ (serum creatinine)$^{-1.094} \times$ age$^{-0.287}$ ($\times 0.739$, if female).[11] CKD was defined as an eGFR <60 ml min^{-1} per 1.73 m^2 and/or the presence of proteinuria.

Study outcome

The primary outcome was determined as the amount of change in the UPCR from the value before commencing treatment to 12 months following commencement of treatment between the two groups. The secondary outcome was the change in blood pressure and eGFR from the value at study commencement to 6 and 12 months following study commencement between the two groups.

Sample size

The planned sample size was 120 cases (60 cases in the LS group and 60 cases in the LS/HCTZ group). With reference to the report by Uzu *et al.*[12] regarding the mean amount of change in urinary protein excretion before and after administration, the difference between CKD patients undergoing administration of ACEI or ARB and those undergoing administration of ACEI or ARB with concomitant use of thiazide was estimated at 0.2 g per day. The standard deviation of the amount of change was surmised to be approximately 0.35 g per day. When a Student's *t*-test was surmised with a difference in mean value of 0.2, standard deviation of 0.35, significance level of 0.05 (two-sided test) and statistical power of 0.80, it was suggested that 50 cases were required for each group. We estimated that 10 to 20% cases would be discontinued/omitted during the study or found to be ineligible following registration, so the target number of cases was set at 60 cases for each group, totaling 120 cases.

Statistical analysis

Statistical analysis was performed using a commercially available software program (JMP statistics 9.0; SAS Institute, Tokyo, Japan). Data are expressed as the mean ± s.d. or as a percentage. A χ^2 test was applied to examine differences between prevalence in the two treatment groups. Data were analyzed based on the random allocation of participants to the treatment group regardless of the content of subsequent drug administration (intention-to-treat analysis). The

mean value of both groups was compared using a Mann–Whitney *U*-test. Repeated measurement analysis of variance was used to evaluate the therapeutic effect against blood pressure, eGFR and uric acid level. $P<0.05$ was determined to be statistically significant.

RESULTS

Baseline characteristics

In this study, 102 cases (85% of the recommended sample size) satisfying the registration criteria were randomly allocated to the LS group ($n=51$) or the LS/HCTZ group ($n=51$) (Figure 2). Patients' backgrounds of the two groups at baseline are illustrated in Table 1, and there was no significant difference between the groups. In the LS group, three patients undergoing treatment were omitted from the protocol (initiation of hemodialysis: $n=1$; long hospitalization for malignant lymphoma: $n=1$; lost of follow-up: $n=1$), and post-operative follow-up as an outpatient could not be carried out for 12 months. Accordingly, these three patients were excluded as study subjects. In the LS/HCTZ group, seven patients were omitted from the protocol (acute worsening of kidney function: $n=3$; withdrawal of consent: $n=3$; skin eruption: $n=1$). However, it was possible to carry out postoperative follow-up as an outpatient for 12 months for all seven patients. Therefore, all 51 patients were included as study subjects.

The drugs taken during the study are shown in Table 2. In the losartan group, there were many cases in which CCB was additionally administered during the course, and the rate of internal use of CCB was significantly higher compared with the LS/HCTZ group.

Changes in clinical blood pressure

There were no differences in SBP or DBP between the two groups during the treatment period (Figure 3). SBP was 125.2 ± 13.3 mm Hg in the LS group and 124.9 ± 15.3 mm Hg in the LS/HCTZ group; DBP was 73.0 ± 9.4 mm Hg in the LS group and 74.1 ± 8.6 mm Hg in the LS/HCTZ group at 12 months following the commencement of treatment, with no significant difference between the two groups. The ratio of patients with blood pressure $<130/80$ mm Hg following 12 months was 50% in the LS group and 47% in the LS/HCTZ group, with no significant difference between the two groups.

Changes in the UPCR

At 6 and 12 months following commencement of the study, the amount of the UPCR decline in the LS/HCTZ group was significantly greater than that in the LS group (6 M: 0.21 ± 0.99 *vs.* -0.54 ± 0.73 g g^{-1} Cr, $P<0.05$; 12 M: 0.02 ± 0.76 *vs.* -0.55 ± 0.71 g g^{-1} Cr, $P<0.05$) (Figure 4a). The relationship between BP reduction and the reduction in proteinuria is not significantly (Figures 4b and c).

Changes in eGFR and uric acid

The eGFR declined slightly more in the LS/HCTZ group compared with the LS group at 6 and 12 months following commencement of treatment; however, there was no significant difference between the two groups (6 M: LS 46.1 ± 23.5 ml min^{-1} per 1.73 m^2, LS/HCTZ 39.6 ± 21.1 ml min^{-1} per 1.73 m^2; 12 M: LS 45.0 ± 23.3 ml min^{-1} per 1.73 m^2, LS/HCTZ 40.5 ± 21.7 ml min^{-1} per 1.73 m^2) (Figure 5). The uric acid level following 12 months was significantly higher in the LS/HCTZ group (6.3 ± 1.3 *vs.* 7.1 ± 1.4 mg dl^{-1}, $P<0.05$) (Figure 6).

DISCUSSION

The results of this study showed that combination therapy with LS/HCTZ led to a greater reduction in proteinuria than treatment with LS alone at the same blood pressure level. This study is the first to provide evidence to support the efficacy of LS/HCTZ combination therapy in patients, independent of antihypertensive effects. This finding suggests that the addition of diuretics constitutes an optimal treatment for patients with CKD under treatment with ARBs and that diuretics exert renoprotective effects through a mechanism independent of blood pressure reduction.

In addition to RAS inhibition, it is believed that strict blood pressure control has a major role in preventing the progression of renal disease.[7,13,14] In this study, there was no difference between SBP and DBP in the two groups, with the average reaching the target blood pressure. In addition, there was no significant difference in the eGFR of both groups throughout the observational period of 1 year.

The following three points may be considered for the mechanism by which LS/HCTZ exhibited a urinary protein reducing effect in this study. The first point is the declining effect on blood pressure with

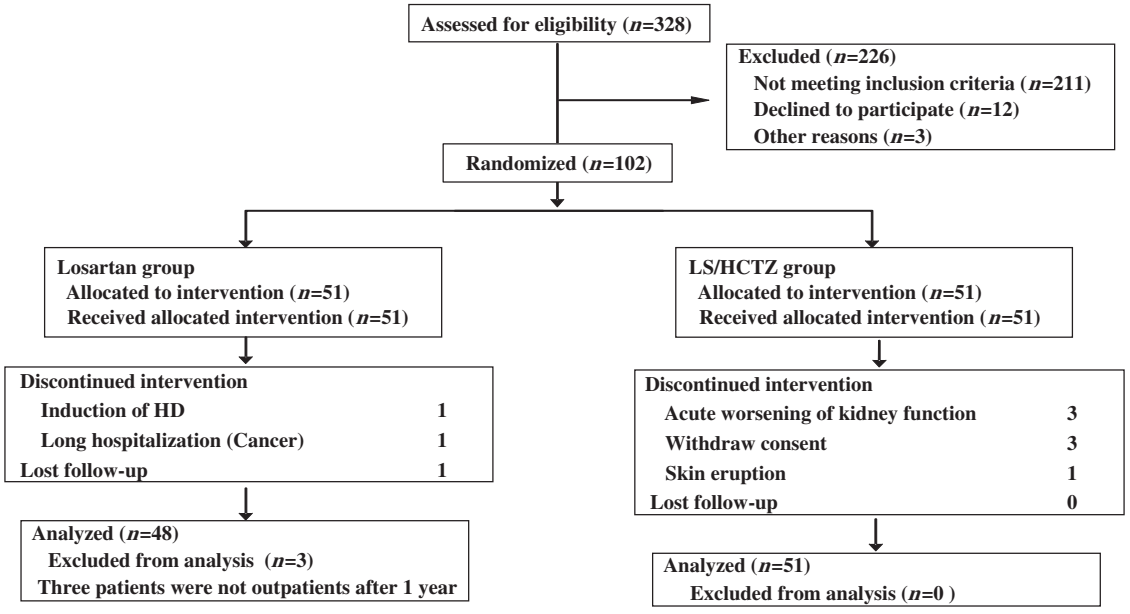

Figure 2 Flow chart of patient enrollment and follow-up. HCTZ, hydrochlorothiazide; HD, hemodialysis; LS, losartan.

Table 1 Baseline characteristics

	Losartan group	*LS/HCTZ group*	P-*value*
Number (*n*)	48	51	
Age (year)	58 ± 12	58 ± 11	0.90
Sex (male/female)	26/22	30/21	0.61
Underlying kidney disease, n *(%)*			
Glomerulonephritis	39 (81)	41 (80)	0.82
Diabetic nephropathy	4 (8)	6 (12)	
Hypertensive nephrosclerosis	5 (11)	5 (8)	
Systolic blood pressure (mm Hg)	132 ± 14	128 ± 13	0.16
Diastolic blood pressure (mm Hg)	77 ± 9	77 ± 12	0.84
Pulse rate (beats min^{-1})	73 ± 10	73 ± 11	0.82
Total protein (g dl^{-1})	6.9 ± 0.6	6.9 ± 0.5	0.55
Albumin (g dl^{-1})	4.0 ± 0.4	3.9 ± 0.4	0.10
HDL-cholesterol (mg dl^{-1})	65 ± 35	61 ± 26	0.73
LDL-cholesterol (mg dl^{-1})	119 ± 35	112 ± 27	0.42
Triglycerides (mg dl^{-1})	157 ± 83	154 ± 76	0.83
Blood urea nitrogen (mg dl^{-1})	24 ± 11	25 ± 11	0.74
Creatinine (mg dl^{-1})	1.4 ± 0.7	1.5 ± 0.7	0.68
Uric acid (mg dl^{-1})	6.6 ± 1.4	6.6 ± 1.3	0.89
Sodium (mEq l^{-1})	138 ± 2	141 ± 2	0.18
Potassium (mEq l^{-1})	4.6 ± 0.5	4.6 ± 0.5	0.82
Chloride (mEq l^{-1})	106 ± 2.6	107 ± 2.7	0.34
Calcium (mg dl^{-1})	9.2 ± 0.4	9.2 ± 0.5	0.70
Phosphate (mg dl^{-1})	3.4 ± 0.6	3.3 ± 0.6	0.72
Aspartate aminotransferase (U l^{-1})	22 ± 6.1	21 ± 5.2	0.89
Alanine aminotransferase (U l^{-1})	20 ± 9.3	17 ± 8.9	0.14
Alkaline phosphatase (U l^{-1})	237 ± 79	225 ± 65	0.65
γ-Glutamyl transpeptidase (U l^{-1})	43 ± 29	38 ± 37	0.14
eGFR (ml min^{-1} per 1.73 m^2)	45.9 ± 25.1	43.8 ± 21.9	0.67
Urinary protein/creatinine ratio (g g^{-1} Cr)	1.80 ± 1.63	1.74 ± 1.40	0.52
Treatment during pretreatment period, n *(%)*			
Ca channel blockers	24 (50)	22 (43)	0.42
β-Blockers	3 (6)	7 (14)	0.43
α-Blockers	2 (4)	1 (2)	0.51
Statins	21 (44)	17 (33)	0.96

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II type 1 receptor blocker; Cr, creatinine; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LS, losartan; HCTZ, hydrochlorothiazide.
Data are presented as mean ± s.d.

Table 2 Medications used during the follow-up period, *n* (%)

	LS group	*LS/HCTZ group*	P-*value*
Ca channel blocker	34 (68)	25 (49)	<0.05
β-Blocker	9 (18)	7 (14)	NS
α-Blocker	3 (6)	1 (2)	NS
Statin	26 (52)	23 (45)	NS

Abbreviations: HCTZ, hydrochlorothiazide; LS, losartan; NS, nonsignificant.

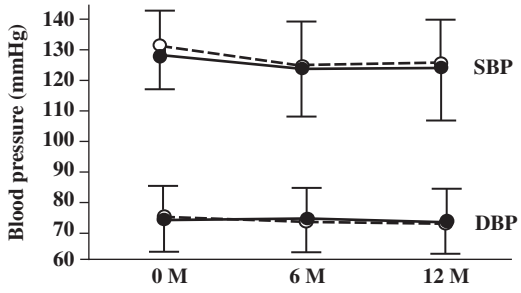

Figure 3 Changes in blood pressure during the study period. Systolic (SBP) and diastolic blood pressure (DBP) levels (mean ± s.d.) at 0, 6 and 12 months after treatment are shown. There were no differences in blood pressure between the losartan (LS) group (open circle and dash line) and the LS/hydrochlorothiazide (HCTZ) group (closed circle and solid line).

respect to nocturnal hypertension. It is thought that diuretics and salt restriction normalizes the circadian rhythm of blood pressure from non-dipper to dipper, thereby reducing the load on the circulatory system and further inhibiting cardiac events by concomitant use with RAS-inhibiting drugs.[15,16] Buter *et al.*[17] reported that the urinary protein reducing the effect of RAS inhibitors was stronger during the day than during the night. It was reported that diuretics and salt restrictions normalize the circadian rhythm of blood pressure from non-dipper to dipper type,[18,19] and the decline in proteinuria by administering diuretics is largely dependent on the decline in nocturnal blood pressure.[12]

The second point is the corrective effect from a state of excessive salt. It has been verified that the salt load promotes local tissue RAS activation in the organ, leading to the development of organ dysfunction.

The third point is that diuretics are reported to have an antioxidant effect, and there is a possibility that renal injury may be improved via a decline in oxidative stress. Skalska *et al.*[20] reported that patients taking diuretics had significantly better antioxidative protection expressed by higher levels of the ferric-reducing ability of plasma.

To date, it has not been suggested that diuretics have an effect on renal protection. Rather, as serum creatinine increases with the administering of diuretics, it was once believed that it is a drug contributing to renal impairment. However, the results from this study suggest the possibility that an increase in serum creatinine and a decline in urinary protein content indicates a decline in intraglomerular pressure owing to diuretics, and that it has an effect on renal protection in the same manner as RAS inhibitors.

According to reports in the GUARD study[8] and the ACCOMPLISH study,[9,10] although HCTZ reduced albuminuria, the decline in eGFR was also very large. Unlike the subjects of the GUARD study and the ACCOMPLISH study, those of the current study were CKD patients showing overt proteinuria in which the eGFR advanced to approximately 40–45 ml min^{-1} per 1.73 m^2. That is, the subjects were cases with a decreased functioning glomerulus count and increased intraglomerular pressure for each nephron unit. In such cases, diuretics used for a relatively long period of time such as HCTZ are believed to be effective for depression management, including management of the quantity of renal protecting body fluid as well as renal protection. It is possible that the result was affected because of Japanese people having a higher salt intake compared with Europeans and Americans. Excessive intake of salt causes excessive body fluid volume, attenuating the effect of ARB. It was hypothesized that excess extracellular fluid was discharged and a synergic effect due to concomitant use of ARB was induced by administering low-dose HCTZ to these patients.

From multiple clinical studies, proteinuria has been proven to be a predictive factor for the advancement of subsequent renal disease. According to a study by Lea *et al.*,[21] baseline proteinuria is independently related to the subsequent decline in GFR. Also, in recent years, albuminuria has been determined as being a risk factor to the cardiovascular system. In the Framingham study, proteinuria increased mortality threefold and was strongly related to other risk

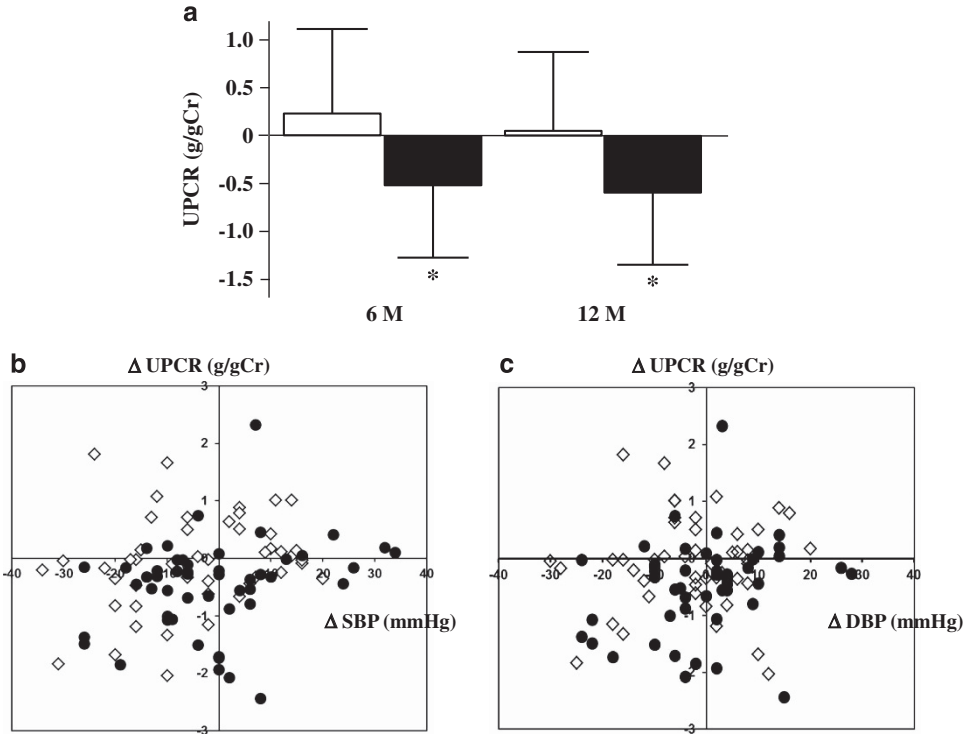

Figure 4 Changes in urinary protein-to-creatinine ratio (UPCR) and relationship between blood pressure (BP) reduction and the reduction in UPCR. (**a**) Mean changes in the UPCR ($g\,g^{-1}$ Cr) from baseline to 6 and 12 months in the losartan (LS) group (open column) and the LS/hydrochlorothiazide (HCTZ) group (closed column) are shown. $^*P<0.05$ *vs.* the LS group. (**b**) The relationship between systolic blood pressure (SBP) reduction (ΔSBP) and the reduction in UPCR (ΔUPCR) from baseline to 12 months was not significant. (**c**) The relationship between diastolic blood pressure (DBP) reduction (ΔDBP) and the reduction in UPCR (ΔUPCR) from baseline to 12 months was not significant. Open square, the LS group; closed circle, the LS/HCTZ group.

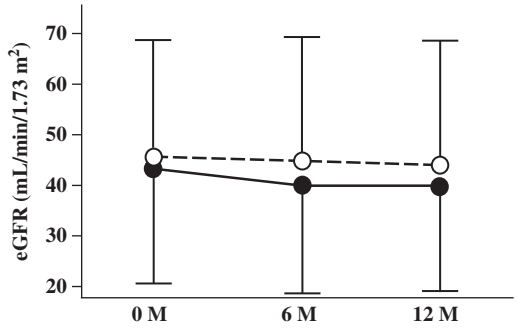

Figure 5 Changes in estimated glomerular filtration rate (eGFR) during the study period. The estimated GFR (mean ± s.d.) values at 0, 6 and 12 months after the start of treatment are shown. There were no differences in eGFR between the losartan (LS) group (open circle and dash line) and the LS/hydrochlorothiazide (HCTZ) group (closed circle and solid line).

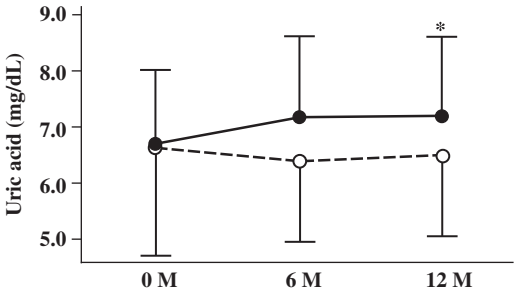

Figure 6 Changes in the uric acid levels during the study period. The uric acid (mean ± s.d.) levels at 0, 6 and 12 months after the start of treatment are shown. There was a significant difference in the uric acid levels at 12 months between the losartan (LS) group (open circle and dash line) and the LS/hydrochlorothiazide (HCTZ) group (closed circle and solid line). $^*P<0.05$ *vs.* the LS group.

factors for cardiovascular diseases.[22] In the subanalysis for the Systolic Hypertension in Europe study, proteinuria was the predictive factor for the all-cause mortality and cardiovascular events.[23] Data for basic and clinical studies to date have exhibited that renal failure and heart failure are suppressed once the proteinuria of CKD patients declines.[24]

In this study, 24-h urine collection was not carried out because it was too inconvenient and troublesome for outpatients. Twenty-four hour urine collection is the gold standard for urinary protein measurement,[25] but it has been reported by several researchers that the UPCR of occasional urine exhibits a strong correlation with 1-day urinary protein excretion.[26,27] The working group for the renal disease prognostic indicator of the National Kidney Foundation also reported that the UPCR of first morning urine or spot urine is a test value suitable at clinical sites when evaluating the proteinuria in patients with renal disease.[28]

In this study, LS/HCTZ was discontinued in three patients in the LS/HCTZ group because of aggravation of renal function. Diuretics have a danger of aggravating renal function in patients on whom sodium restriction is being carried out or in patients with declined body fluid, so sufficient attention is required.

Our study has several limitations. Blood pressure was measured at an outpatient clinic, and no investigation into home blood pressure and 24-h ambulatory blood pressure monitoring was carried out. Therefore, the improvement effect of LS/HCTZ against nocturnal hypertension on the subjects of this study has not been evaluated and

proved. Also, the sample size was 85% of the initially planned number of cases. However, we could find a significant difference in the primary outcome with the number of cases used in this study, because the difference in the amount of urinary protein was more than expected. Finally, the rate of use of CCB was significantly higher in the LS group than in the LS/HCTZ group. However, this bias did not appear to affect this result because the difference in the amount of urinary protein remained significant, even after adjustment for use of CCB.

In conclusion, in CKD patients with hypertension and overt proteinuria, the effect of reducing urinary protein was higher in the LS/HCTZ group than that in the LS group even when blood pressure was equivalently controlled. We believe that the concomitant use of ARB and thiazide diuretics should be considered for CKD patients with hypertension and overt proteinuria.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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