as iron or calcium deposits, bone, or vessel flow voids; and (7) a clinical history excluding any traumatic diffuse axonal injury. A cerebral infarction was defined as a focal hypointense lesion with a hyperintense rim on the fluid-attenuated inversion recovery sequence images, with a corresponding hyperintensity on the T2-weighted images and corresponding hypointensity on the T1-weighted images. Cerebral infarctions with no plausible clinical history were defined as asymptomatic cerebral infarctions (ACIs).

MRI Protocol

The image protocol included T1-weighted, fluid-attenuated inversion recovery, T2-weighted, and GRE T2*-weighted MRI. Imaging of the brain was performed on three 1.5 T MRI scanners at our hospital: the Signa Excite (General Electric Medical Systems, Waukesha, Wisconsin), Magnetom Sonata (Siemens Medical Solutions, Munich, Germany), and Achieva MR (Philips Healthcare, Bothell, Washington) scanners. The GRE sequence parameters for the Signa Excite were as follows: 22 axial images; field of view, 220 mm; slice thickness, 5.5 mm; interslice gap, 1.0 mm; 256 × 256 matrix; echo time, 20 ms; repetition time, 640 ms; and flip angle, 20°. For the Magnetom Sonata, the parameters were as follows: 22 axial images; repetition time, 600 ms; echo time, 18 ms; slice thickness, 5.5 mm; interslice gap, 1.0 mm; field of view, 175 × 200 mm; and flip angle, 20°. For the Achieva MR scanner, the parameters were as follows: repetition time, 700 ms; echo time, 20 ms; slice thickness, 5.5 mm; interslice gap, 1.0 mm; field of view, 210 mm; and flip angle, 20°.

Statistical Analysis

Statistical analyses were performed using SPSS version 11.0 software (SPSS Inc, Chicago, IL). The Mann–Whitney *U* test, Student *t* test, or Fisher exact test were used for the group comparisons. The proportions of the initial CMB ownership with the $CHADS_2$ score were compared using the Mann–Whitney *U* test. The cumulative event-free rates were estimated by the Kaplan–Meier curves, and the curves of the different groups were compared using the log-rank test. The hazard ratio (HR) and 95% confidence interval (CI) of the ACIs and CMBs during the follow-up period were obtained using a multivariate Cox proportional hazards analysis with a forced entry method. In this regard, the clinical variables with a value of *P* less than .10 in the univariate analysis were entered into the multivariate analysis. Also, we did not include the $CHADS_2$ score, but did include each component with a value of *P* less than .10 in the multivariate analysis, because the $CHADS_2$ score and its components have a multicollinear relationship. Finally, we considered a value of *P* less than .05 to be statistically significant.

Results

Cross-sectional Study

The 131 patients with AF included 93 (71.0%) male patients. There were 112 Group C patients consisting of 60 (53.6%) male patients without AF or symptomatic cerebral infarctions and included 24 patients with Parkinson disease, 15 with hypertension, 12 with spinocerebellar degeneration, 5 with Alzheimer disease, 5 with dizziness, 4 with blepharospasms, 4 with headaches, 3 with essential tremors, 3 with progressive supranuclear palsy, and 37 with other neurologic diseases. The mean ages of the 2 groups did not differ significantly (AF 69.4 ± 9.2 versus control 69.0 ± 9.5).

The baseline proportion of CMBs in the patients with AF was 40 (30.5%) and was significantly greater than that in the Group C patients (21, 18.8%; OR, 1.91; 95% CI, 1.05-3.46; $P = .038$) (Table 1). The prevalences of baseline CMBs in the patients with AF according to the $CHADS_2$ score were 17.2% (0 points), 22.5% (1 point), 44.4% (2 points), 36.0% (3 points), and 100% (4 points), respectively. Thus, higher $CHADS_2$ scores yielded a higher detection rate of CMBs ($P = .01$) (Fig 1). On the contrary, the prevalences of baseline CMBs in the Group C patients according to the $CHADS_2$ score were 12.8% (0 points), 17.9% (1 point), 22.7% (2 points), 66.7% (3 points), and 100% (4 points), respectively. Although the higher $CHADS_2$ scores tended to yield a higher detection rate of CMBs ($P = .05$), there was no significant difference (Fig 2).

Longitudinal Study

Seventy-seven patients underwent over 3 yearly MRI scans (35 patients for 3 times, 33 patients for 4 times, and 9 patients for 5 times, respectively). The patients were divided into 2 groups, 24 with CMBs at baseline (AF + CMBs, mean follow-up duration: 2.54 ± .66 years) and 53 without CMBs at baseline (AF − CMBs, 2.71 ± .69 years). When divided into 2 groups according

Table 1. *Profiles of patients in groups AF and control*

Baseline characteristic /risk factor	AF (N = 131)	Control (N = 112)	*P*
Age	69.4 ± 9.2	69.0 ± 9.5	.75
Male sex	93 (71.0)	60 (53.6)	.005
Hypertension	73 (55.7)	43 (38.4)	.01
Diabetes mellitus	36 (27.5)	16 (14.3)	.02
Statin	44 (33.6)	17 (15.7)	.001
Warfarin	86 (64.1)	0 (.0)	
Antiplatelets	59 (45.0)	12 (10.7)	<.001
Baseline CMBs	40 (30.5)	21 (18.8)	.04
Baseline ACIs	42 (32.1)	29 (25.9)	.32

Abbreviations: ACIs, asymptomatic cerebral infarctions; AF, atrial fibrillation; CMBs, cerebral microbleeds.

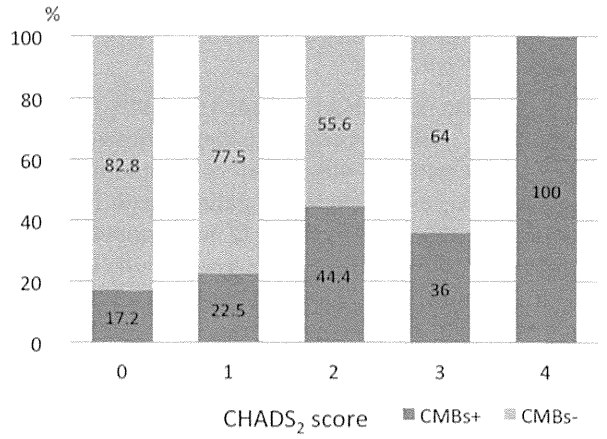

Figure 1. *Proportion positive for CMBs using the $CHADS_2$ score in the AF group. A higher $CHADS_2$ score yields a greater detection rate of CMBs (P = .01). Abbreviations: AF, atrial fibrillation; $CHADS_2$, 1 point for congestive heart failure, hypertension, age 75 years, and diabetes, and 2 points for previous stroke/transient ischemic attack (TIA); CMBs, cerebral microbleeds.*

to whether ACIs developed, significant differences in the incidence of hypertension, diabetes mellitus, and baseline CMBs were observed between the 2 groups (Table 2). Similarly, when divided into 2 groups according to whether CMBs developed, there were significant differences in the age, $CHADS_2$ score, baseline CMBs, and baseline ACIs (Table 2). The Kaplan–Meier curve showed that the development of an ACI was associated with the baseline presence of a CMB ($P = .004$) (Fig 3). The development of new CMBs also was significantly associated with the baseline presence of CMBs ($P < .001$, Fig 4). No symptomatic cerebral infarctions were observed in either group during the follow-up duration.

A univariate analysis demonstrated that the $CHADS_2$ score, baseline CMBs, hypertension, and diabetes mellitus were significant predictors of the future development of an ACI. We applied the $CHADS_2$ score as separated items into a multivariate analysis for a detailed investigation. A multivariate Cox regression analysis showed that only the baseline presence of CMBs was independently related to the new development of an ACI (HR, 5.41; 95% CI, 1.03-28.43; $P = .046$) (Table 3).

Regarding the concern for the future development of CMBs, a univariate analysis demonstrated that the $CHADS_2$ score, baseline CMBs, baseline ACIs, and age more than 75 were significant predictors. A multivariate Cox regression analysis showed that baseline CMBs (HR, 6.27; 95% CI, 1.43-27.5 6; $P = .015$) and ACIs (HR, 14.14; 95% CI, 2.56-78.24; $P = .002$) were significantly related to the new development of CMBs (Table 3).

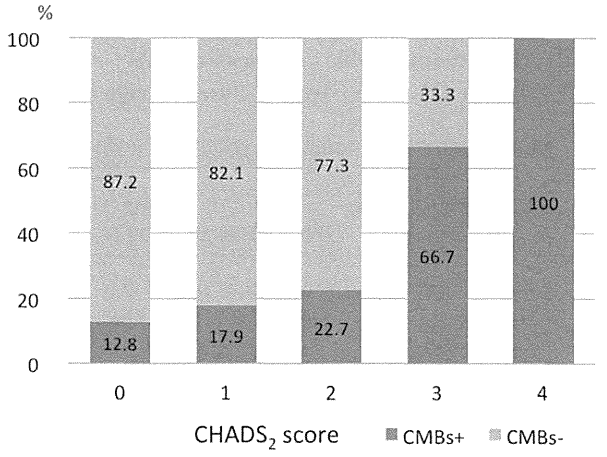

Figure 2. *Proportion positive for CMBs using the $CHADS_2$ score in Group C. Although a higher $CHADS_2$ score appeared to yield a greater detection rate of CMBs, there was no significant difference (P = .05). Abbreviations: $CHADS_2$, 1 point for congestive heart failure, hypertension, age 75 years, and diabetes, and 2 points for previous stroke/transient ischemic attack (TIA); CMBs, cerebral microbleeds.*

Discussion

The present findings demonstrated that both the relatively high prevalence and causative background for ischemic strokes associated with CMBs in patients with AF and without a history of symptomatic stroke.

Relationship between AF and CMBs

One of the most important findings of this study was that CMBs tend to be detected more frequently in patients with AF. CMBs have been considered to represent a perivascular accumulation of hemosiderin-containing macrophages as a corollary of extravasated erythrocytes from cerebral small vessels.[10] Therefore, it may suggest that endothelial dysfunction and blood–brain barrier impairment are prone to complications in cerebral small vessels under conditions involving AF. Ovbiagele et al[11] suggested that CMBs might represent the presence and severity of background heart disease. Even if congestive heart failure is not evident, other $CHADS_2$ components may play an important role in aggravating the cardiac condition followed by cardiogenic embolisms and CMBs resulting from the vulnerability of cerebral small vessels. The present study demonstrated that a higher $CHADS_2$ score yielded a higher prevalence of CMBs, thereby supporting the previously mentioned hypothesis. However, we have to take account of the fact that patients with AF tended to receive anticoagulation and antiplatelets more frequently than patients without AF for the prevention of thromboembolisms, and anticoagulation and antiplatelets themselves could induce CMBs.

A cross-sectional study showed another important result. The higher $CHADS_2$ scores tended to yield a greater but an insignificant detection rate of CMBs in the group of patients without AF. It is difficult to judge which would be more responsible for the generation of CMBs between AF and the $CHADS_2$ score, because both influence each other from their occurrence to their progression. However, in the present study, we pointed out

Table 2. *Profiles of patients assorted by the development of ACIs or CMBs*

	Development of ACIs			Development of CMBs		
Baseline characteristic/risk factor	(+) N = 8	(−) N = 69	*P*	(+) N = 11	(−) N = 66	*P*
Male sex	4 (50.0%)	49 (71.0%)	.25	3 (27.3%)	45 (68.2%)	1.00
Congestive heart failure	5 (62.5%)	24 (34.8%)	.15	6 (54.5%)	23 (34.8%)	.31
Hypertension	2 (25.0%)	43 (62.3%)	.06	4 (36.4%)	41 (62.1%)	.18
Age > 75 y	4 (50.0%)	22 (31.9%)	.43	8 (72.7%)	18 (27.3%)	.006
Diabetes mellitus	4 (50.0%)	13 (18.8%)	.07	4 (36.4%)	13 (19.7%)	.25
$CHADS_2$ score	2.36 ± 1.41	1.58 ± 1.23	.10	2.73 ± .79	1.48 ± 1.24	.002
CKD	4 (50.0%)	27 (39.1%)	.71	5 (45.5%)	26 (39.4%)	.75
Dyslipidemia	6 (75.0%)	47 (68.1%)	1.00	7 (63.6%)	46 (69.7%)	.73
Statin	4 (50.0%)	29 (42.0%)	.72	4 (36.4%)	28 (42.4%)	1.00
Warfarin	4 (50.0%)	47 (68.1%)	.43	9 (82.8%)	42 (63.6%)	.32
Antiplatelets	5 (62.5%)	36 (52.2%)	.72	4 (36.4%)	37 (56.1%)	.33
Baseline CMBs	6 (75.0%)	18 (26.1%)	.010	8 (72.7%)	16 (24.2%)	.003
Baseline ACIs	4 (50.0%)	18 (26.1%)	.21	9 (82.8%)	13 (19.7%)	<.001

Abbreviations: ACIs, asymptomatic cerebral infarctions; AF, atrial fibrillation; $CHADS_2$, 1 point for congestive heart failure, hypertension, age 75 years, and diabetes, and 2 points for previous stroke/transient ischemic attack (TIA); CKD, chronic kidney disease; CMBs, cerebral microbleeds; HR, hazard ratio.

the possibility that not only higher $CHADS_2$ scores but also AF itself performs a crucial role in generating CMBs.

Relationship between CMBs and ACIs

Another important finding of this study is that the patients with AF with the presence of CMBs in the baseline T2*-weighted MRI tended to suffer significantly from new ACIs in the following MRI; however, when interpreting the results of this research, the small number of cases needs to be considered. It is well known that the prevalence of an ACI in a patient with AF is over 4 times greater than that in patients with sinus rhythm.[12] It has been suggested that CMBs initiate the occurrence of cerebral hemorrhages in subjects with a history of cerebral hemorrhage[13] and ischemia.[14] However, a longitudinal study on CMBs in patients with AF without any history of cerebrovascular disease has never been reported. It seems reasonable that some pathophysiological mechanism of AF itself enhances the vulnerability of cerebral small vessels.

In the present study, although the $CHADS_2$ score was the strongest risk factor for the development of CMBs, the $CHADS_2$ score did not reach a significant level for the development of ACIs. Lone AF results from an accumulation of cardiovascular risk factors. Each component of the $CHADS_2$ score is a cardiovascular risk factor. In patients with AF, the higher $CHADS_2$ score implies a more advanced microangiopathy as we have previously discussed. CMBs are one of the most pure representational forms of microangiopathy. Therefore, the patients who develop CMBs later should have a higher $CHADS_2$ score. On the contrary, cerebral infarctions in AF result

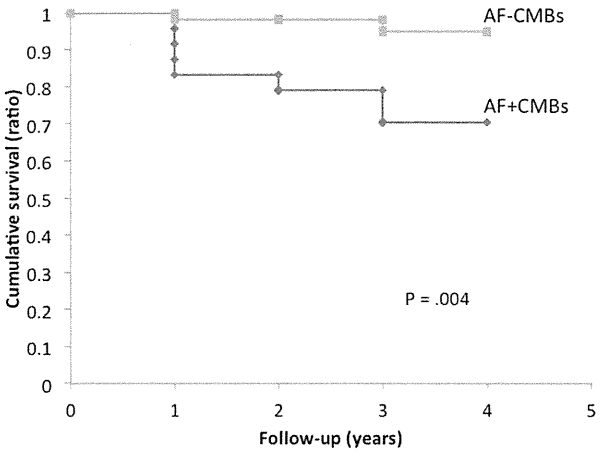

Figure 3. *Kaplan–Meier curve of a subsequent asymptomatic cerebral infarction according to the baseline presence of CMBs (log-rank test, P = .015). Abbreviations: AF, atrial fibrillation; CMBs, cerebral microbleeds.*

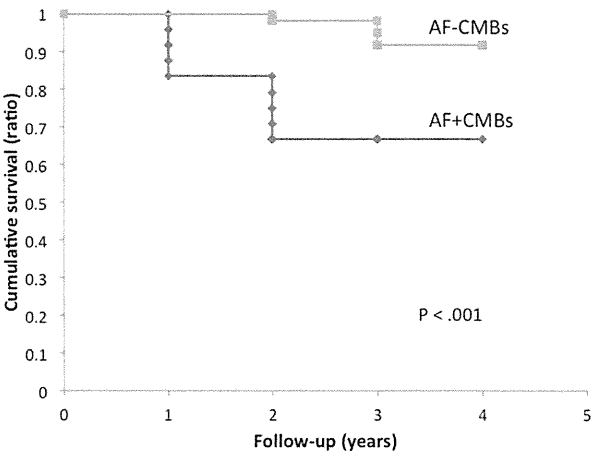

Figure 4. *Kaplan–Meier curve of subsequent CMBs according to the baseline presence of CMBs (log-rank test, P < .001). Abbreviations: AF, atrial fibrillation; CMBs, cerebral microbleeds.*

Table 3. *Univariate and multivariate analyses of 77 patients with AF with more than 3 times of MRI. The table is divided into 2 broad parts on the basis of the development of ACIs or CMBs*

	Development of ACIs				Development of CMBs			
	Univariate		Multivariate		Univariate		Multivariate	
Variates	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)
Male sex	.27	2.18 (.54-8.73)			.82	.86 (.23-3.23)		
Congestive heart failure	.14	2.95 (.70-12.35)			.21	2.14 (.65-7.02)		
Hypertension	.08	.24 (.05-1.17)	.09	.25 (.05-1.26)	.13	.39 (.11-1.34)		
Age > 75 y	.26	2.22 (.55-8.93)			.008	6.09 (1.61-23.00)	.10	3.22 (.82-12.67)
Diabetes mellitus	.06	3.72 (.93-14.87)	.15	2.86 (.68-12.00)	.25	2.06 (.60-7.04)		
$CHADS_2$ score	.09	1.6 (.94-2.56)			.003	1.89 (1.24-2.87)		
CKD	.50	1.61 (.40-6.48)			.65	1.32 (.40-4.33)		
Dyslipidemia	.75	1.3 (.26-6.43)			.69	.78 (.23-2.66)		
Statin	.63	1.41 (.35-5.64)			.79	1.18 (.36-3.87)		
Warfarin	.29	.47 (.12-1.89)			.31	2.22 (.48-10.27)		
Antiplatelets	.65	1.4 (.33-5.85)			.24	.48 (.14-1.64)		
Baseline CMBs	.01	7.62 (1.53-37.98)	.046	5.41 (1.03-28.43)	.004	6.93 (1.83-26.19)	.015	6.27 (1.43-27.56)
Baseline ACIs	.11	3.13 (.79-12.76)			.001	14.92 (3.16-70.49)	.002	14.14 (2.56-78.24)

Abbreviations: ACIs, asymptomatic cerebral infarctions; AF, atrial fibrillation; $CHADS_2$, 1 point for congestive heart failure, hypertension, age 75 years, and diabetes, and 2 points for previous stroke/transient ischemic attack (TIA); CKD, chronic kidney disease; CMBs, cerebral microbleeds; HR, hazard ratio.

not only from microangiopathy but also from cardioembolisms. Hypercoagulability and a decreased blood flow might occupy an important place in the pathogenesis of cerebral infarctions in AF. For this reason, we speculated that the $CHADS_2$ score was especially important for the development of CMBs. Actually, the HR of warfarin for the development of CMBs was 2.22, but on the contrary for the ACIs it was .47; however, both HRs were insignificant.

Several reports have suggested that certain inflammatory mechanisms are involved in ischemic strokes[15-17] and CMBs[18,19] as well as AF.[20-23] If such inflammatory mechanisms aggravate the vulnerability and hyperpermeability of the endothelium, rupture of the endothelium and erythrocyte leakage into the perivascular space would result in CMB formation. We speculated that CMBs and infarctions of perforating branches may have a common pathogenesis evoked by AF itself, but we did not check the change in the inflammatory markers in the present study. Further surveillance is required to clarify this.

This study has several limitations. Firstly, the results of this research have limited effectiveness because of the small number of cases. It was difficult to follow-up the patients with AF over a period of years. To determine the association between CMBs and AF with sufficient statistical power, more patients must be studied for a long duration as a large clinical trial. Secondly, in the cross-sectional study, we did not confirm a detailed comparative review of the clinical features between the AF and control groups, because the control group resulted in gathering many kinds of different neurologic diseases. In addition, the mechanistic cause of AF is multifactorial, for example, hypertension, heart failure, aging, male sex, and so on. As a result, the number of male patients in Group AF was significantly greater than that in the control group but at least the mean ages of the 2 groups did not differ significantly. Thirdly, no symptomatic cerebral infarctions were newly observed during less than 5 years of follow-up in the present study. Longer-term follow-up is required to elucidate whether the patients with AF with the presence of CMBs are prone to suffer any type of cerebral infarction. Finally, we used 3 types of MRI systems over 5-years of follow-up in the present study. Ideally, all the imaging should have been processed by a single MRI system. Accordingly, we very cautiously performed a comparative review of the MRI images.

Conclusions

To the best of our knowledge, this is the first report that investigated local cerebral vessel disease in patients with AF without a history of cerebral infarction using GRE T2*-weighted MRI and pursued the findings longitudinally. The proportion of CMBs in patients with AF was significantly greater than that in the control patients. The patients with AF with the presence of CMBs in the baseline MRI were accompanied by a significant increase in the CMBs and ACIs.

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Drug Target Insights

Febuxostat for Hyperuricemia in Patients with Advanced Chronic Kidney Disease

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ABSTRACT: Febuxostat is a nonpurine xanthine oxidase (XO) inhibitor, which recently received marketing approval. However, information regarding the experience with this agent among advanced chronic kidney disease (CKD) patients is limited. In the current study, we investigated the effects of oral febuxostat in patients with advanced CKD with asymptomatic hyperuricemia. We demonstrated, for the first time, that not only the serum levels of uric acid (UA) but also those of 8-hydroxydeoxyguanosine, an oxidative stress marker, were significantly reduced after six months of febuxostat treatment, with no adverse events. These results encouraged us to pursue further investigations regarding the clinical impact of lowering the serum UA levels with febuxostat in advanced CKD patients in terms of concomitantly reducing oxidative stress via the blockade of XO. More detailed studies with a larger number of subjects and assessments of the effects of multiple factors affecting hyperuricemia, such as age, sex, and dietary habits, would shed light on the therapeutic challenges of treating asymptomatic hyperuricemia in patients with various stages of CKD.

KEYWORDS: febuxostat, chronic kidney disease, hemodialysis, uric acid, oxidative stress

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Introduction

Hyperuricemia, defined as a serum urate level exceeding the limit of solubility, mirrors supersaturation of the extracellular fluid with urate, and predisposes affected subjects to gout, which is characterized by the tissue deposition of monosodium urate crystals, although it is a necessary but not a substantial factor for the development of the disease.[1] The current urate-lowering strategies include reducing the urate production with xanthine oxidase (XO) inhibitors and accelerating the urinary excretion of uric acid (UA) with uricosuric agents.[2,3] Uricosuric agents, such as probenecid and benzbromarone, may have limited effectiveness in patients with reduced renal function.[3,4] The purine analog XO inhibitor, allopurinol, has remained widely prescribed for the treatment of hyperuricemia, but requires dose adjustment in subjects with renal impairment, which may lead to a reduced benefit.[2,3,5,6]

Febuxostat, a nonpurine XO inhibitor that recently received marketing approval, has been focused on as an alternative option for the treatment of hyperuricemia in patients with chronic kidney disease (CKD) because it undergoes hepatic metabolism and may require less dose adjustment in association with the renal function.[6,7] Moreover, several lines of evidence have focused on the blockade of XO activity as a potential therapeutic strategy for various other kinds of oxidative stress-mediated tissue and vascular injuries.[8,9] However, information regarding the experience with this therapeutic agent among patients with advanced CKD is limited.[7] In this regard, the current study investigated the effects of febuxostat

in patients with advanced CKD with hyperuricemia in terms of the reduction of the serum UA levels and the longitudinal changes in several serum indicators for oxidative stress.

Materials and Methods

Seventeen patients on chronic hemodialysis (HD) treatment who had serum UA levels above 8.0 mg/dL and who were not receiving anti-hyperuricemic agents participated in the study. All subjects had oliguria or anuria. The subjects had to be in stable condition, and they had no history of active liver diseases or any other significant medical status, no change in diuretics or steroid therapy within one month of study enrollment and were not chronic users of any nonsteroidal anti-inflammatory drugs. The usual medications, such as anti-hypertensive agents, erythropoietin, and phosphate binders, were continued during the study period. Sex was not considered. The exclusion criteria were as follows: age <20 years or >90 years, type I diabetes mellitus or type II diabetes mellitus with poor glucose control (glycosylated hemoglobin >9% at the start of the observation period), treatment with mercaptopurine hydrate or azathiopurine, pregnancy, and any medical or surgical conditions that made patients unsuitable for this study as judged by the attending physician. All patients were assigned to oral febuxostat and entered the six-month treatment period from July through August 2012, during which they initially received febuxostat 10 mg orally once daily in the morning. The target serum UA level was <6.0 mg/dL, and the dose of febuxostat was titrated or increased up to a maximum of 40 mg/day.

The blood pressure (BP) was measured before all HD sessions, and the data regarding the systolic BP and diastolic BP were the average of each value on the last HD day of the week. The blood samples were obtained from vascular access, including arteriovenous fistulas and arteriovenous grafts, before HD sessions. The hemoglobin (Hb), hematocrit (Hct), platelet count (Plt), serum levels of UA, blood urea nitrogen (BUN), creatinine (Cr), sodium (Na), chloride (Cl), potassium (K), calcium (Ca), inorganic phosphate (Pi), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were measured at baseline (week 0) and every four weeks during the observation period. The serum levels of 8-hydroxydeoxyguanosine (8-OHdG), 3-nitrotyrosine-modified proteins (3-NT), and protein carbonyls were determined at baseline and at weeks 4, 12, and 24 during the treatment period. The serum levels of 8-OHdG were measured by an enzyme-linked immunosorbent assay (ELISA) as described previously.[10] The ELISA method was also used to measure the serum levels of 3-NT (Japan Institute for the Control of Aging, Nikken SEIL Co, Shizuoka, Japan) and protein carbonyls (BioCell Co, Auckland, New Zealand). This study was performed in accordance with the Declaration of Helsinki and was approved by the medical ethics committee of Jichi Medical University, and all patients included in the present study provided their informed consent.

The data were expressed either as the number of participants or as the percentage (%) of the study population. The remaining data were expressed as the means ± standard deviation (SD), or as medians and interquartile ranges (IR) for variables with a skewed distribution. A repeated measures analysis of variance combined with Fisher's protected least significant difference test for normal distributions and the Kruskal–Wallis test with Dunn's method for skewed distributions were used to compare the time course data, when appropriate. Values of $P < 0.05$ were considered to be statistically significant. The statistical analyses were performed using the SigmaPlot 12 software program for Windows (Systat Software, Inc., San Jose, CA) unless otherwise stated.

Results and Discussion

The demographic profiles of the 17 patients included in the present study are summarized in Table 1. No subjects had a history of gouty attacks. The patients had been treated with chronic HD for a median of four years. The causes of advanced CKD included diabetic nephropathy, chronic glomerulonephritis, hypertensive nephrosclerosis, and polycystic kidney disease. All subjects were on the optimum tolerated medical management. Febuxostat lowered the serum UA levels (8.9 ± 1.0 at baseline) significantly from one month after the initiation of the treatment (Fig. 1), and the target serum UA level (<6.0 mg/dL) was achieved in 12 patients (70.5%) after one month of treatment, compared to 13 (76.4%) and 14 (82.3%) patients after three and six months of treatment, respectively.

After six months, 16 subjects were still under the treatment with oral febuxostat (10 mg/day), and there was

Table 1. Demographic profiles of the patients at the start of the study.

DEMOGRAPHIC CHARACTERISTICS	
Age (years)	64 ± 10
Sex (male/female)	15/2
HD duration (years)	6.4 ± 5.7
UNDERLYING CAUSES OF CKD, N (%)	
Diabetic nephropathy	9 (53)
Chronic glomerulonephritis	3 (18)
Hypertensive nephrosclerosis	3 (18)
Polycystic kidney disease	2 (12)
MEDICATIONS, N (%)	
Calcium channel antagonist(s)	7 (41)
Angiotensin-converting-enzyme inhibitor	1 (6)
Angiotensin receptor blocker(s)	7 (41)
Renin inhibitor	1 (6)
Other anti-hypertensive agent(s)	4 (24)
Loop diuretic(s)	3 (18)

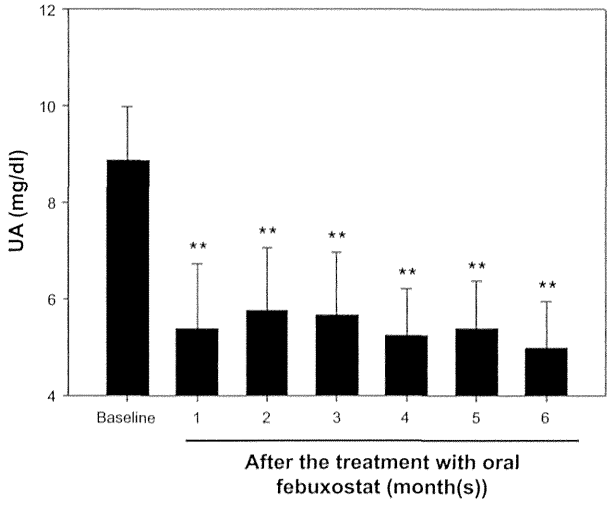

Figure 1. The serum UA levels before and after the initiation of the treatment with oral febuxostat. After six months of febuxostat treatment, the serum UA levels were significantly decreased from those at baseline. All patients were entered into the six-month treatment period from July through August 2012. Note that the decrease in the serum UA levels was already significant after one month treatment with febuxostat.
Notes: $N = 17$, $^{**}P < 0.01$ versus baseline.

one subject who was being treated with a reduced dose of 5 mg/day. Also, only two patients were treated with the agent at a dose of above 10 mg/day at that point, one with 20 mg/day and one with 40 mg/day. All doses of febuxostat were well tolerated by the patients with no withdrawals because of side effects or allergic reactions. Although there was a significant increase in the systolic BP values obtained at five and six months compared to those observed at baseline, no significant changes in diastolic BP, Hb, Hct, Plt, BUN, or the serum levels of Cr, Na, Cl, K, Ca, Pi, AST, ALT, or LDH were noted during the observation period (Table 2). There were similar trends in the serum levels of protein carbonyls and 3-NT, while the serum 8-OHdG levels measured after six months were significantly lower than those at the baseline (Table 3). No patients experienced symptoms of gouty arthritis, including joint pain, swelling, or redness,[2] during the observation period.

The treatment of patients with asymptomatic hyperuricemia (a serum UA level higher than 8 mg/dL) with urate-lowering agents has been recommended and applied in Japan,[7,11] while the appropriate dose of febuxostat among subjects with advanced CKD has not yet been established. The current observations suggest that even relatively low doses of febuxostat, which is approved at a dose of 40 to 60 mg/day as the standard dose for the treatment of hyperuricemia with or without gouty arthritis in Japan, may also work effectively among chronic HD patients for reducing the serum UA to a level that has been arbitrarily proposed as a therapeutic target for hyperuricemia.[7,11,12] The validity of the indications for urate-lowering agents among overall subjects with asymptomatic

Table 2. Changes in clinical parameters during the observation period.

	AFTER THE INITIATION OF ORAL FEBUXOSTAT TREATMENT						*P* VALUE
	BASELINE	1 MONTH	2 MONTHS	3 MONTHS	4 MONTHS	5 MONTHS	6 MONTHS	
Systolic BP (mmHg)	141 ± 20	137 ± 16	141 ± 29	141 ± 16	142 ± 21	148 ± 21*	149 ± 19*	<0.001
Diastolic BP (mmHg)	74 ± 11	73 ± 9	73 ± 10	76 ± 10	76 ± 12	74 ± 11	73 ± 11	0.186
Hb (g/dL)	10.7 ± 1.0	10.8 ± 1.0	10.9 ± 1.1	10.8 ± 1.6	10.9 ± 1.3	10.9 ± 1.3	10.6 ± 1.0	0.989
Hct (%)	33.9 ± 3.1	33.9 ± 3.2	33.5 ± 3.4	34.1 ± 3.4	34.0 ± 4.3	34.0 ± 3.6	33.1 ± 2.9	0.976
Plt ($\times 10^4/\mu l$)	16.8 (IR: 14.2–22.7)	15.6 (IR: 13.6–25.1)	17.8 (IR: 14.2–24.6)	18.4 (IR: 14.9–25.2)	17.7 (IR: 12.8–23.1)	16.7 (IR: 12.8–21.6)	18.3 (IR: 11.7–21.8)	0.887
BUN (mg/dL)	62.1 ± 11.7	64.8 ± 18.6	65.1 ± 15.8	63.2 ± 14.1	64.7 ± 19.1	67.0 ± 21.4	69.9 ± 16.5	0.886
Cr (mg/dL)	11.4 ± 1.9	11.8 ± 2.0	11.8 ± 1.9	11.8 ± 2.3	11.9 ± 2.2	11.5 ± 2.2	11.5 ± 2.2	0.991
Na (mmol/l)	138 ± 4	139 ± 4	140 ± 4	139 ± 4	139 ± 4	139 ± 3	138 ± 2	0.805
K (mmol/l)	4.9 ± 0.9	4.9 ± 0.8	5.0 ± 1.0	5.0 ± 0.9	4.9 ± 0.7	5.0 ± 0.8	5.0 ± 0.9	0.999
Cl (mmol/l)	102 ± 5	103 ± 4	103 ± 4	103 ± 4	103 ± 5	102 ± 4	103 ± 3	0.93
Ca (mg/dL)	8.8 ± 0.8	9.0 ± 0.9	8.8 ± 0.6	8.8 ± 0.6	8.9 ± 0.7	9.1 ± 0.8	9.0 ± 0.9	0.852
Pi (mg/dL)	4.5 ± 1.3	5.1 ± 1.2	5.3 ± 1.4	5.0 ± 0.9	5.1 ± 1.5	5.6 ± 1.5	4.9 ± 1.0	0.296
AST (U/l)	16.5 ± 15.8	18.0 ± 15.4	15.0 ± 6.7	15.0 ± 6.4	17.2 ± 7.5	18.4 ± 8.4	18.3 ± 13.6	0.938
ALT (U/l)	17.9 ± 17.6	18.8 ± 16.7	15.2 ± 9.4	14.4 ± 8.3	14.0 ± 8.0	17.5 ± 10.4	19.4 ± 18.2	0.857
LDH (U/l)	179 ± 40	189 ± 39	179 ± 28	178 ± 26	180 ± 30	191 ± 45	188 ± 48	0.923

Note: $^*P < 0.05$ versus baseline.

Table 3. The serum levels of several oxidative stress markers before and after the initiation of the treatment with oral febuxostat.

	AFTER THE INITIATION OF ORAL FEBUXOSTAT TREATMENT				*P* VALUE
	BASELINE	1 MONTH	3 MONTHS	6 MONTHS	
Protein carbonyls (nmol/mg protein)	0.07 (IR: 0.03–0.12)	0.10 (IR: 0.06–0.14)	0.07 (IR: 0.06–0.15)	0.05 (IR: 0.03–0.11)	0.329
3-NT (nM)	30.4 (IR: 25.2–40.4)	33.2 (IR: 25.5–42.2)	37.3 (IR: 25.3–45.3)	28.7 (IR: 24.7–43.9)	0.928
8-OHdG (ng/ml)	1.64 (IR: 1.24–2.95)	2.51 (IR: 1.24–4.74)	1.05 (IR: 0.84–1.87)	0.54 (IR: 0.204–1.16)*	0.002

Note: *$P < 0.05$ versus baseline.

hyperuricemia remains to be delineated, and clinicians should bear in mind that the prevalence of refractory gout and/or gouty tophi is much lower in Japan in comparison to that in the United States and Europe, where negative opinions regarding pharmaceutical interventions predominate.[11–14] One may argue that the clinical benefit of using urate-lowering agents requires careful evaluation, especially in subjects on chronic HD treatment, since an incipient gouty attack is quite rare in hyperuricemic long-term HD patients, and the frequency of gouty arthritis has been shown to decrease after the initiation of a periodic HD program in advanced CKD subjects.[15,16] Moreover, a significant association between higher serum UA levels and lower mortality, which may be dependent on the favorable nutritional status, has been demonstrated in the HD population.[17] Otherwise, it may be necessary to focus on the fact that some of the reactive oxygen species are produced as a by-product of urate formation through a XO-dependent pathway and the pharmacological nature of febuxostat, which is characterized by a higher bioavailability and a more potent blockade of XO activity than the traditional XO inhibitor allopurinol.[1,2,8,18] Indeed, the superior potency of febuxostat to allopurinol for the inhibition of reactive oxygen synthesis has been demonstrated in several reports,[19,20] and the serum UA levels could be used as a surrogate indicator of the XO activity.

In the current study, 8-OHdG, 3-NT, and protein carbonyls, which have been included in the list of the most common oxidative stress biomarkers in various settings,[20,21] were used as the indices of the condition of the present patients. Numerous processes other than the XO-mediated pathway have been implicated in the oxidative stress among the subjects with CKD.[21,22] In addition, it has been shown that chronic HD treatment also leads to excessive radical production and impairment of the anti-oxidant capacity.[23] Despite the lack of qualitative information regarding the significance of individual oxidative stress markers,[24] we feel that it is reasonable to consider that 8-OHdG, but not 3-NT and protein carbonyls, may help to detect the inhibitory effects of febuxostat on XO-mediated oxidative stress among the chronic HD patients. Although the precise role of febuxostat in reducing the serum levels of 8-OHdG remains to be delineated, our findings suggest that the generation of 8-OHdG may be more dependent on XO than that of 3-NT or protein carbonyls. On the other hand, it has been proposed that the XO inhibitors may be utilized as adjunctive anti-hypertensive agents in some subsets of hyperuricemic and hypertensive subjects associated with or without advanced CKD,[25,26] while we found that the systolic BP levels after five and six months of treatment with febuxostat were higher than those at baseline, despite the titration of anti-hypertensive agents corresponding to the context. We have no explanation for this discrepancy; however, a seasonal bias may have been involved. Indeed, it has been reported that the systolic BP shows seasonal changes in chronic HD patients, with peak BP noted in the winter.[27]

Finally, the number of patients included in the present series was quite small, thus implying that this study may be statistically underpowered or that the clinical parameters may have been overestimated. As such, our findings should be interpreted with caution. Nevertheless, our results encourage us to pursue further investigations regarding the clinical impact of lowering the serum UA level with febuxostat in chronic HD patients in terms of determining the appropriate dose of the agent as well as concomitantly reducing oxidative stress by blocking XO. Indeed, despite the comparable serum levels of low-density lipoprotein (LDL) between baseline and week 24, we found that the serum levels of the oxidative form of LDL, another oxidative stress marker,[20] at week 24 were significantly decreased compared to those observed at baseline among the 12 subjects who led us to determine the serum levels of these parameters (data not shown). Obviously, more detailed studies with a larger number of subjects and assessments of the effects of multiple factors affecting hyperuricemia, such as age, sex, and dietary habits,[2,14] would shed light on the therapeutic challenges of treating asymptomatic hyperuricemia in patients with various stages of CKD.[28]

Author Contributions

TA drafted the manuscript. YM, CI, OI, ST, and YW made contributions to the acquisition of the clinical data. EK and DN provided a detailed review of the contents and structure of the manuscript, resulting in significant changes to the original document. All authors have read and approved the final manuscript.

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Clinical Implication of the Renin-angiotensin-aldosterone Blockers in Chronic Kidney Disease Undergoing Hemodialysis

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Abstract: The renin-angiotensin-aldosterone system (RAAS) blockers have been widely used in chronic kidney disease patients undergoing hemodialysis; however, whether RAAS blockers have beneficial effects for cardiovascular disease in those patients has not been fully defined. This review focuses on the effects of RAAS blockers in chronic kidney disease undergoing hemodialysis for cardiovascular disease.

Keywords: Hemodialysis, clinical study, renin, angiotensin I, angiotensin II, aldosterone, angiotensin-converting enzyme inhibitor, angiotensin receptor blockers, direct renin inhibitor, cardiovascular disease.

INTRODUCTION

The cardiovascular diseases (CVD) are often complicated in chronic kidney disease undergoing hemodialysis (HD). CVD are main factor which affects the prognosis of HD patients [1-3]. Accumulated evidence suggested that antihypertensive therapy may have beneficial effects for the development of CVD in HD patients [4, 5]. The renin-angiotensin-aldosterone system (RAAS) has been reported to contribute to the hypertension, and to increase chronic inflammation and oxidative stress on vascular endothelium that may result in CVD in HD patients [6-8]. These lines of evidence suggest that RAAS blockers may have beneficial effects to prevent CVD and improve prognosis in HD patients; however, their effects have not been fully defined. This review focuses on the clinical studies of RAAS blockers in HD patients in terms of CVD.

Clinical Studies of RAAS Blockers in HD Patients

The clinical studies that investigated the effects of RAAS blockers for the CVD in HD patients are summarized in Table **1**.

Angiotensin-converting Enzyme Inhibitors (ACEIs)

Angiotensin-converting enzyme inhibitors (ACEIs) block the conversion of angiotensisn I (Ang I) to angiotensisn II (Ang II) which leads the constriction of blood vessels, and increase blood pressure. Tradolapril and captopril have been reported to be effective for control hypertension in HD patients [9, 10]. Zheng *et al.* reported tradopril (2-8 mg/thrice a week) after HD session with atenolol and/or amlodipine (they were given if the patients had any member of these classes drugs as their daily regimen) significantly decrease blood pressure (from 122.2±7.1 / 75.3±10.4 mmHg to 116.4±11.6 / 70.4±11.4 mmHg) in ten HD patients [9]. Wauterd *et al.* reported that the effect of captopril (25 to 200 mg) for hypertension in eight HD patients that showed resistant hypertension for ultrafiltration and conventional antihypertensive therapy [10]. They reported that four HD patients decreased blood pressure at normal level with captopril alone and the four remaining patients also showed significant blood pressure reduction by the combination of captopril and salt removal by replacement of 1-2 liters of ultrafiltrate by an equal volume of 5% dextrose without a significant change in body weight [10]. These studies showed that ACEIs has beneficial effects for hypertension in HD patients. In addition, several studies reported that ACEIs showed cardio protective effects in HD patients as follows. The perindopril (2-4 mg after each HD session) and imidapril (2.5 mg/day) have been reported to significantly reduce left ventricular mass in HD patients compared with control group that were treated with a calcium channel antagonist or placebo respectively [11, 12]. In addition to that, this cardio protect effect by these ACEIs was suggested to be independent of blood pressure lowering effect because there was no difference in terms of the change of blood pressure between ACEIs treatment groups and control groups [11, 12].

On the other hands, there are several reports that ACEIs have no beneficial effect for CVD in HD patients. Zannad *et al.* reported that no significant benefit was found in fosinopril (5-20 mg/day) for the prevention of CVD (cardiovascular death, resuscitated death, nonfatal stroke, heart failure, myocardial infarction or revascularization) in HD patients [13]. Chang *et al.* reported that there were no significant associations among ACEIs use and total mortality and hospitalization due to CVD in HD patients [14]. Furthermore, ACEIs use was associated with a higher risk of hospitalization due to heart failure [14]. These contradict results required further large scale clinical trials to investigate the effects of ACEIs for CVD and in HD patients.

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Table1. Clinical studies of RAAS blockers in HD patients.

RAAS Blockers	References	Number	Duration (month)	Intervention		Results			
				Treatment	Control	Treatment ⊿SBP/DBP (mmHg)	Control ⊿SBP/DBP (mmHg)	Treatment CVD	Control CVD
ACEIs	Zheng *et al.* (9)	10	0.5-2	tradopril (2-8mg/ TIW)		-5.8 / -4.9			
	Wauterd *et al.* (10)	8	5	captopril (25-200mg/ 2 day)		-45 / -29			
	London *et al.* (11)	24	12	perindopril (2-4mg/ after each HD)	nitrendipine (20-40mg/ after each HD) placebo	-27 / -15	-20 / -10	-70 g (LVM)	NS
	Matsumoto *et al.* (12) 30		6	imidapril (2.5mg / day)		NS	NS	-36 g (LVM)	NS
	Zannad *et al.* (13)		397	24	Fosinopril (5-20mg / day)		placebo + conventional therapy	No significant benefit for fosinopril	
	Chang *et al.* (14)		1846	16-52	ACE inhibitor +CCB, β-blocker	CCB, β-blocker		ACE inhibitor: Hazard ratio 1.41	
ARBs	Saracho *et al.* (15)	406	6	losartan		-11 / -5			
	Shibasaki *et al.* (16)	24	30	losartan (50mg / day)	amlodipine (5mg/day), enalapril (5mg/day)	-11 (MBP) amlodipine:-11(MBP) enalapril: -11 (MBP)		-24.7% (LVMI)	amlodipine: -10.5% (LVMI) enalapril: -11.2% (LVMI)
	Kannno *et al.* (17)	12	24	losartan (100mg / TIW) + existing CCB, α-blocker or centrally acting agents	Placebo+ existing CCB, α-blocker or centrally acting agents			-23 g/m2 (LVMI)	NS
	Takahashi *et al.* (18)	19	80	candesartan (4-8mg / day)+ ACE inhibitor + CCB, α-blocker or centrally acting agents	placebo+ACE inhibitor+CCB, α-blocker or centrally acting agents	NS	NS	Treatment group 16.3 % vs. control group 45.9 %	
	Onishi *et al.*(19)	17	3	Irbesartan (50-100 mg)		-15.5/-6.7			

Table 1. Contd.....

RAAS Blockers	References	Number	Duration	Intervention		Results			
			(month)	Treatment	Control	Treatment	Control	Treatment	Control
						⊿SBP/DBP	⊿SBP/DBP	CVD	CVD
	Suzuki *et al.* (20)	366	36	valsartan(160 mg / day), candesartan(12 mg / day) or losartan (100 mg / day) + CCB, α-blocker or centrally acting agents	CCB, α-blocker or centrally acting agents	-14 / -1	-16 / -4	Treatment group 19 % vs. control group 33 %	
ACEIs/ARBs	Bajaj *et al.* (21)	1950	30	ACEIs or ARBs	CCB or statins	Primary outcome (mortality and cardiovascular events) was no significant difference among ACEIs/ARBs group (HR 0.95) and statin group (HR 1.08) compared with CCB group			
	Iseki *et al.* (22)	469	42	Olmesartan (10-40 mg)	no ACEIs and ARBs	Primary outcome (mortality and cardiovascular events) was no significant difference between			
						olmesartan group (HR 1.00) compared with no ACEI/ARB group			
Direct renin inhibitor	Morishita *et al.* (24)	30	2	Aliskiren (150 mg / day) + existing ACE inhibitor, ARB, CCB, α-blocker or centrally acting agents		-15 / -5			
	Ishimitsu *et al.*(25)	23	6	Aliskiren (150mg)		-8 (SBP)			
	Takenaka *et al.*(26)	30	6	Alsikiren (150-300 mg)		-5 (SBP)			
Aldosteron-receptor blocker	Gross *et al.* (31)	8	0.5	spironolactone (50 mg / twice daily)		-11 (SBP)			
	Shavit et.al. (32)	8		eplerenone (25mg / twice daily)		-13 (SBP)			

SBP: systolic blood pressure, DBP: diastolic blood pressure, CVD: cardio vascular disease, LVM: left ventricular mass, LVMI: left ventricular mass index, NS, no siginicant, CCB calcium channnel blocker, MBP mean blood pressure

Angiotensin Receptor Blockers (ARBs) in HD Patients

Angiotensin receptor blockers (ARBs) works to block the activation of Ang II by competitive antagonism of angiotensin II receptor type1 (AT1 receptor). Losartan has been reported to reduce blood pressure at before and after HD in hypertensive HD patients in large size clinical study [15]. Losartan reported to reduce left ventricular mass index after compared with amlodipine (calcium channel antagonist) or an enalapril (ACEI), although similar blood pressure lowering were detected in all three groups [16]. Another studies also reported that losartan (100 mg/thrice a week) reduced left ventricular hypertrophy in 24 HD patients whereas a placebo group showed no change [17]. These results suggested that this beneficial effect of losartan for cardio protection was independent of a blood pressure lowering effect. The effects of another ARBs for CVD also have been reported. Candesartan (4-8 mg/day) reduced cardiovascular events and mortality compared with placebo after in HD patients [18]. In this study, the brain natriuretic peptide (BNP) levels were significantly increased in the control group but not in

the candesartan group [18]. Irbesartan (50-100mg/day) significantly reduced blood pressure in stable maintaining HD patients [19]. Suzuki *et al.* reported that several ARBs (valsartan, candesartan or losartan) treatment was reduced CVD compared with no ARB treatment group for HD patients in each group during a three-year observation period [20]. There were 19% fatal or nonfatal CVD events in the ARBs group and 33% in the no ARB group [20]. Blood pressure did not differ between the ARBs group and the no ARB group. After adjustment for age, sex, diabetes, and systolic blood pressure, treatment with an ARBs was independently associated with reduced fatal and nonfatal CVD events [20]. These lines of evidence demonstrated that ARBs are effective to control blood pressure and prevent CVD in HD patients. A certain level of cardio protective effects of ARBs may be independent from a blood pressure lowering effect in HD patients.

On the other hands, recently several clinical studies have been reported that ARBs have no beneficial effect for CVD and mortality in HD patients. Bajaj *et al.* reported that ARBs or ACEIs treatment was not associated with an overall reduction in CVD events compared with calcium channel blockers or statins treatment groups in elderly HD patients during the 2.4 years observation period [21]. Iseki *et al.* also reported that olmesartan treatment did not alter mortality and CVD events compared with non ACEIs and ARBs group in hypertensive HD patients during 3.5 years follow-up period [22]. These contradict results required a large size and long term clinical studies to investigate the effects of ARBs in terms of the prevention of CVD events in HD patients.

Direct Renin Inhibitor in HD Patinets

An oral direct renin inhibitor; aliskiren inhibits renin activity [23]. Although renin level will increase due to negative feedback of aliskiren, Ang I, Ang II level and PRA will decrease. Little was known the effects of aliskiren in HD patients. Previously, we reported on an blood pressure lowering effect and potential CVD protective effect of aliskiren in hypertensive HD patients [24]. In that study, aliskiren significantly reduced blood pressure in HD patients [24]. In addition to that, aliskiren reduced the surrogate markers for CVD such as BNP, high-sensitivity CRP (hs-CRP), and an oxidative stress marker [24]. Isimitsu *et al.* also reported that alsikiren (150mg/day) significantly reduced blood pressure in maintaining HD patients [25]. Takenaka *et al.* reported that the alsikiren reduced morning blood pressure measured at home in HD patients with diabetic nephropathy [26]. These lines of evidence suggested that alsikiren has beneficial effects for blood pressure control in HD patients.

It should be noted that the combination of aliskiren and other class RAAS blockers should be careful because of the severe advert effects. ALTITUDE study to investigate the effects of aliskiren added to ACEIs or ARBs in patients at high risk for CVD and with diabetes and renal impairment was suspended because more adverse effects such as stroke, renal impairment, hyperkalemia and hypotension were observed in patients who received aliskiren than in patients who received a placebo [27-29]. We also reported that when we followed up the HD patients who had been received aliskiren treatment in the previous study for 20 months, high rate (44%) of discontinuation of aliskiren owing to symptomatic hypotension was observed [30]. In that study, most patients had been received alsikiren added on their existing antihypertensives including AECIs and ARBs [30]. Taken together, the careful observation for blood pressure change is required for aliskiren treatment in hypertensive HD patients especially the combination with the other class of RAAS blockers such as ACEIs or ARBs, and further studies will be required to establish the effects of aliskiren in HD patients.

Aldosterone-receptor Blockers in HD Patients

Aldosterone-receptor blockers are receptor antagonists at the mineralocorticoid receptor. Antagonism of these receptors inhibits sodium resorption in the collecting duct of the kidney. There are a few studies to investigate the efficacy of aldosterone-receptor blocker in HD patients. Gross *et al.* reported spironolactone (50 mg/ twice daily) significantly reduced pre-dialysis systolic blood pressure after 2 weeks in 8 oligo-anuric HD patients [31]. Shavit et la. reported that eplerenone (20 mg/ twice daily) significantly reduced systolic blood pressure after 4 weeks by in oligo-anuric HD patients [32]. These lines of evidence suggested that beneficial effect of aldosterone-receptor blockers as antihypertensive drugs in HD patients; however, the additional large size and long term studies will be required to confirm the efficacy of aldosterone-receptor blockers in HD patients.

The Combination Therapy of RAAS Blockades in HD Patients

There are a few clinical studies to investigate the combination therapy of RAAS blockers in HD patients. Chan *et al.* reported that initiated on combined ACE and ARB therapy were at increased risk of CVD compared with initiated on an ARB and non-ACEI after adjustment for risk factors in large size clinical study [33]. These results suggested that combination of ARB and ACEI may not have a beneficial effect on CVD in HD patients.

Adverse Effects of RAAS Blockers

ACEIs showed several adverse effects in HD patients. ACEIs may suppress erythropoiesis and induce resistance to erythropoietin [34]. Several possible mechanisms have been described: Ang II can stimulate erythroid progenitor cell growth and that ACEIs can inhibit this [35], ACEIs increase plasma levels of a natural stem cell regulator which inhibits the recruitment of pluripotent haemopoietic stem cells, hence inhibit erythroid growth [36], ACEIs have been shown to reduce production of interleukin-12 which can stimulate erythropoiesis [37]. Occasionally, ACEIs may cause anaphylactoid reactions with AN69 dialysis membrane by increase serum bradykinin level [38-40]. Therefore it is better to avoid the combination of AN69 membranes with ACEIs in HD patients. Hyperkalemia, which is a frequent concern in HD patients, is the primary danger from RAAS blocking medications. The blockade RAAS leads to a decrease in aldosterone levels. Since aldosterone has a central role for the excretion of potassium, the RAAS blocker can cause retention of potassium. Several clinical trials of ACEIs, ARBs, a renin inhibitor and an aldosterone receptor-blocker in HD patients tracked potassium levels [13, 18, 20,

24, 31]. Significant trend for increased hyperkalemia by these RAAS blockers in HD patients was not observed in these trials. Although careful and periodical monitoring of plasma potassium level is required, these results suggested that the risk of hyperkalemia by RAAS blocking in HD patients is small.

SUMMARY

RAAS blockers have been reported to have beneficial effects for blood pressure control and CVD to some extent in HD patients. However, the effects of those have not been fully established. Hence, the choice of the RAAS inhibitors in the treatment of HD patients should be carefully determined with close monitoring blood pressure. Further high-quality studies are still required to confirm the effects of RAAS blockade on CVD in HD patients.

CONFLICT OF INTEREST

None declared

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None declared

ABBREVIATION

ACEIs = Angiotensin-converting enzyme inhibitors

ARBs = Angiotensin receptor blockers

AngI = angiotensin I

Ang II = angiotensin II

BNP = brain natriuretic peptide

CVD = cardiovascular disease

HD = hemodialysis

hs-CRP = high-sensitivity CRP

PRA = plasam renin activity

RAAS = renin-angiotensin-aldosterone system

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Original Article

Factors Associated with Remission and/or Regression of Microalbuminuria in Type 2 Diabetes Mellitus

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The aim of this study was to clarify the factors associated with the remission and/or regression of microalbuminuria in Japanese patients with type 2 diabetes mellitus. We retrospectively analyzed the data of 130 patients with type 2 diabetes mellitus with microalbuminuria for 2-6 years (3.39 ± 1.31 years). Remission was defined as improving from microalbuminuria to normoalbuminuria using the albumin/creatinine ratio (ACR), and regression of microalbuminuria was defined as a decrease in ACR of 50% or more from baseline. Progression of microalbuminuria was defined as progressing from microalbuminuria to overt proteinuria during the follow-up period. Among 130 patients with type 2 diabetes mellitus with microalbuminuria, 57 and 13 patients were defined as having remission and regression, respectively, while 26 patients progressed to overt proteinuria. Sex (female), higher HDL cholesterol and lower HbA1c were determinant factors associated with remission/regression of microalbuminuria by logistic regression analysis. Lower systolic blood pressure (SBP) was also correlated with remission/regression, but not at a significant level. These results suggest that proper control of blood glucose, BP and lipid profiles may be associated with remission and/or regression of type 2 diabetes mellitus with microalbuminuria in clinical practice.

Key words: microalbuminuria, type 2 diabetes mellitus, remission, regression

The number of diabetes mellitus patients is dramatically increasing in Japan and has become public health challenge. It is well known that diabetic nephropathy is one of the most serious complications of diabetes mellitus. More than 300,000 patients in Japan undergo hemodialysis, and about 44% of those just starting hemodialysis in 2012 were affected by diabetes mellitus [1]. In addition, diabetic nephropathy is an independent risk factor for cardiovascular disease, and has a serious impact on quality of life and health care costs [2-7]. The earliest known manifestation of diabetic nephropathy is the presence of small amounts of albumin in the urine, called microalbuminuria [8]. In our country, more than 40% of patients with type 2 diabetes mellitus have microalbuminuria [9]. Recommended treatment of diabetic nephropathy is as follows: 1) tight control of blood

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glucose, 2) tight control of blood pressure (BP), 3) suppression of the renin-angiotensin system (RAS) using angiotensin-converting-enzyme (ACE) inhibitors or angiotensin-receptor blockers (ARBs), 4) control of lipid profiles, 5) diet therapy, and 6) lifestyle modification (including smoking cessation) [9]. In patients with type 2 diabetes mellitus, the course of renal dysfunction is more heterogeneous, and the natural progression less well characterized than in patients with type 1 diabetic nephropathy [10]. Furthermore, for a wide variety of treatment methods, the impact of each treatment on preventing and improving diabetic nephropathy in patients with type 2 diabetes mellitus has not been fully examined in clinical practice.

In the present study, we evaluated the remission/regression vs. progression of microalbuminuria in Japanese patients with type 2 diabetes mellitus in a single clinical practice. In addition, we clarified the factors that are associated with remission and/or regression of microalbuminuria.

Subjects and Methods

Subjects. We retrospectively collected the data of 130 subjects who met the following criteria: (1) diagnosed as having type 2 diabetes mellitus in accordance with the criteria of the Japanese Diabetes Society [11] and the World Health Organization [12] at the Department of Medicine, Okayama University Hospital between January 2006 and December 2009; (2) having microalbuminuria by at least 2 measurements of albumin/creatinine ratio (ACR) in a spot urine sample, and their diabetic nephropathy status was determined; (3) having no complicating cancer, liver disease, collagen disease, or nondiabetic kidney disease confirmed by renal biopsy; and (4) having received follow-up physical examinations at least every two months, while undergoing measurements of ACR in a spot urine sample at least once a year for 2–6 years.

All participants received treatment based on the standard strategies for diabetes mellitus, hypertension, and dyslipidemia during these periods.

This study was approved by the Ethics Committee on Epidemiological Studies of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences.

Assessment of urinary albumin excretion. The albumin excretion rate was estimated on the basis of the ACR in spot urine samples, as previously described [13–15]. The levels of albumin excreted in each measurement of ACR were classified as normoalbuminuria (ACR < 30 mg/g creatinine), microalbuminuria ($30 \leq$ ACR < 300 mg/g creatinine), and overt proteinuria (ACR ≥ 300 mg/g creatinine).

Criteria for remission, regression and progression of microalbuminuria. We applied the criteria of previous reports for remission, regression and progression of microalbuminuria [16, 17]. Remission of microalbuminuria was defined as returning to normoalbuminuria during the follow-up period; regression of microalbuminuria was defined as a decrease in ACR of 50% or more from baseline. Otherwise, progression of microalbuminuria was defined as progressing from microalbuminuria to overt proteinuria during the follow-up period.

Clinical parameters. All data were retrospectively obtained from electronic charts. Body mass index (BMI) was the weight in kilograms divided by the square of the height in meters. The estimated Glomerular Filtration Rate (eGFR) was calculated using the following equation: eGFR (ml/min/1.73 m^2) $= 194 \times \mathrm{Cr}^{-1.094} \times \mathrm{Age}^{-0.287} \times 0.739$ (a constant derived specifically for women) [18]. Among antihypertensive drugs, RAS blockade drugs including ACE inhibitors or ARBs and other antihypertensive drugs were separately recorded and analyzed.

Statistical analysis. Values are expressed as means ± standard deviations (SDs) for continuous variables. The participants were classified by the course of microalbuminuria, remission and/or regression, no change and progression. One-way analysis of variance (ANOVA) and Tukey's F test were used to compare among 3 groups. Participants were also classified as being with or without remission/regression of microalbuminuria during the follow-up period. Comparisons between these 2 groups was performed using Chi-squared tests for categorical variables and unpaired t tests for continuous variables. Univariate and multivariate analyses were also performed using a logistic regression model.

To investigate the importance of each clinical practice recommendation, we dichotomized the levels of HbA1c, BP, and lipid profiles as salutary or nonsalutary. The salutary levels were defined as follows: HbA1c $< 6.5\%$, BP $< 130/80$ mmHg, and lipid profiles

< 150 mg/dl for triglycerides, < 120 mg/dl for LDL cholesterol, and ≥ 40 mg/dl for HDL cholesterol, according to the clinical practice recommendations of the Japanese Diabetes Society [19]. We then coded each follow-up period of observation on a scale from 0 to 3 according to the number of the 3 factors with a salutary level, and calculated hazard ratios.

All statistical analyses were performed using the SPSS 21.0 software program for Windows. We selected p values < 0.05 as the threshold of statistical significance in all tests.

Results

During the follow-up period (average of 3.4 years), among 130 type-2 diabetes mellitus patients with microalbuminuria, 57 patients (43.8%) demonstrated remission of microalbuminuria, 13 patients (10.0%) demonstrated regression of microalbuminuria, and 26 patients (20.0%) progressed to overt proteinuria. The remission/regression rate was higher than that of progression.

The clinical characteristics of the patients at baseline according to the course of microalbuminuria are summarized in Table 1. Among participants with remission and/or regression of microalbuminuria, the proportions of males and alcohol drinkers were significantly lower than those in other categories. In participants with progression of microalbuminuria, systolic BP, ACR and diabetic retinopathy were significantly higher than those in other participants. In participants with remission/regression of microalbuminuria, HDL cholesterol was significantly higher than that in participants with progression of microalbuminuria.

Next, we compared the clinical characteristics

Table 1 Clinical characteristics of the study participants at baseline according to the course of microalbuminuria

	Remission and/or Regression	No change	Progression
Number of participants: n (%)	70 (53.8)	34 (26.2)	26 (20.0)
Male sex: n (%)	30 (42.9)[ab]	24 (70.6)	20 (76.9)
Age (years)	59.9 ± 14.3	62.0 ± 13.8	64.3 ± 11.5
Duration of diabetes (years)	9.6 ± 7.9	10.7 ± 10.1	14.2 ± 11.2
Current smoking: n (%)	16 (27.6)	8 (27.6)	12 (50.5)
Alcohol drinker: n (%)	12 (20.7)[ab]	15 (50.0)	11 (45.8)
Body mass index (kg/m^2)	25.3 ± 4.5	25.7 ± 4.7	24.6 ± 4.1
Systolic blood pressure (mmHg)	128.9 ± 14.7[b]	132.1 ± 11.4[b]	141.2 ± 15.9
Diastolic blood pressure (mmHg)	74.7 ± 9.5	74.9 ± 11.6	79.0 ± 12.4
HbA1c (%)	7.5 ± 1.4	7.4 ± 1.0	7.7 ± 1.5
Plasma glucose (mg/dl)	168.0 ± 58.1	181.5 ± 60.2	178.2 ± 49.6
BUN (mg/dl)	17.2 ± 8.5	17.2 ± 6.6	18.3 ± 4.9
Serum creatinine (mg/dl)	0.81 ± 0.73	0.86 ± 0.25	0.88 ± 0.32
Estimated Glomerular Filtration Rate (ml/min/1.73m^2)	79.6 ± 28.0	70.1 ± 20.3	71.8 ± 25.2
ACR (mg/g creatinine)	69.6 ± 61.6[b]	79.0 ± 73.9[b]	152.9 ± 107.2
Uric acid (mg/dl)	5.2 ± 2.0	5.6 ± 1.4	5.5 ± 1.4
Total cholesterol (mg/dl)	198.0 ± 34.1	204.7 ± 31.8	184.1 ± 38.9
Triglycerides (mg/dl)	147.4 ± 90.7	178.9 ± 113.7	158.9 ± 157.8
HDL cholesterol (mg/dl)	60.1 ± 15.9[b]	54.0 ± 16.1	50.4 ± 12.6
LDL cholesterol (mg/dl)	111.7 ± 28.6	117.3 ± 29.6	103.9 ± 24.9
Diabetic neuropathy: n (%)	26 (39.4)	13 (40.6)	15 (60.0)
Diabetic retinopathy: n (%)	23 (33.8)[b]	8 (25.8)[b]	16 (61.5)
IHD: n (%)	8 (11.4)	4 (11.8)	4 (15.4)
CVD: n (%)	3 (4.3)	2 (5.9)	2 (7.7)
PAD: n (%)	2 (2.9)	2 (5.9)	1 (3.8)
Use of ACE inhibitor or ARBs: n (%)	30 (42.9)	18 (52.9)	16 (61.5)
Use of Statin: n (%)	20 (28.6)	12 (35.5)	6 (23.1)

Data are means ± SD unless otherwise indicated. IHD, ischemic heart disease. CVD, cerebral vascular disorder. PAD, peripheral arterial disease.
[a]$p < 0.05$ versus no change. [b]$p < 0.05$ versus progression.

between patients with and without remission/regression in the follow-up period. In the univariate analysis, significant differences of plasma glucose, HbA1c, creatinine and BUN were noted between the 2 groups (Table 2). To adjust for confounding factors such as duration of diabetes, total cholesterol and smoking habits by logistic regression analysis, we found that the female sex [odds ratio (OR): 4.34, (95% confidence interval (CI): 1.70–11.12), $p=0.002$], higher HDL cholesterol (≥ 50 mg/dl) [OR: 3.65, (95% CI: 1.06–9.88), $p=0.031$] and lower HbA1c ($\leq 6.0\%$) [OR: 5.61, (95% CI: 1.13–27.85), $p=0.035$] were independently associated with remission/regression of microalbuminuria (Table 3), whereas lower SBP (≤ 130 mmHg) [OR: 2.66, (95% CI: 0.83–7.23), $p=0.122$] was weakly associated with remission/regression of microalbuminuria, *i.e.*, not at a significant level.

Table 2 Clinical characteristics of the study participants at follow-up according to the presence or absence of remission and/or regression of microalbuminuria

	Remission and/or Regression	No Remission and/or Regression	*p* value
Mean follow-up time (years)	2.8 ± 1.1	4.1 ± 1.6	<0.001*
Body mass index (kg/m^2)	25.4 ± 4.3	25.3 ± 4.4	0.893
Systolic blood pressure (mmHg)	129.3 ± 11.6	131.1 ± 12.0	0.398
Diastolic blood pressure (mmHg)	73.6 ± 9.1	73.6 ± 11.0	0.991
HbA1c (%)	6.7 ± 0.8	7.2 ± 1.2	0.023*
Plasma glucose (mg/dl)	151.1 ± 32.2	168.8 ± 52.4	0.028*
BUN (mg/dl)	16.3 ± 4.6	18.7 ± 6.4	0.020*
Serum creatinine (mg/dl)	0.75 ± 0.20	0.91 ± 0.29	<0.001*
Estimated Glomerular Filtration Rate (ml/min/1.73m^2)	71.4 ± 21.2	64.9 ± 19.7	0.079
Uric acid (mg/dl)	5.4 ± 1.3	5.7 ± 1.3	0.215
Total cholesterol (mg/dl)	190.5 ± 25.1	192.2 ± 43.3	0.793
Triglycerides (mg/dl)	134.9 ± 71.4	161.0 ± 117.9	0.131
HDL cholesterol (mg/dl)	55.6 ± 14.7	51.6 ± 12.8	0.117
LDL cholesterol (mg/dl)	109.5 ± 19.9	105.2 ± 22.1	0.254
Use of ACE inhibitor or ARBs: n (%)	39 (55.7)	41 (68.3)	0.153
Use of Statin: n (%)	24 (34.3)	31 (51.7)	0.074

Data are means ± SD unless otherwise indicated. *$p < 0.05$.

Table 3 The ORs of factors associated with the remisson and/or regression of microalbuminuria with the logistic regrsssion model

Factor	Adjusted odds ratio	95% confidence interval	*p* value
Sex (female)	4.34	1.70–11.12	0.002*
HDL cholesterol (mg/dl)			0.031*
HDL < 40	1	ref.	
40 ≤ HDL < 50	1.10	0.23–3.06	
50 ≤ HDL < 60	3.65	1.06–9.88	
60 ≤ HDL	4.17	1.20–11.53	
HbA1c (%)			0.035*
7.0 < HbA1c	1	ref.	
6.5 < HbA1c ≤ 7.0	3.53	0.67–18.63	
6.0 < HbA1c ≤ 6.5	4.26	0.83–21.92	
HbA1c ≤ 6.0	5.61	1.13–27.85	
Systolic blood pressure (mmHg)			0.122
140 < SBP	1	ref.	
130 < SBP ≤ 140	1.42	0.51–3.76	
SBP ≤ 130	2.66	0.83–7.23	

The multivariate model was adjusted for BMI, duration of diabetes, total cholesterol, smoking habits, and use of ACE inhibitor or ARBs. Ref., reference category. *$p<0.05$.