the change in the urinary albumin/creatinine ratio after 12 months of treatment.

Conclusions The present trial is expected to clarify whether the sympatholytic CCB azelnidipine is a beneficial second-line choice for RAS inhibitor-treated hypertensive patients with CKD, such as diabetic nephropathy.

Key words Calcium channel blocker · Urinary albumin · Diabetic nephropathy · Hypertension · Renin-angiotensin system inhibitor

Introduction

Hypertension, which is often associated with chronic kidney disease (CKD), accelerates the progression of kidney injury and associated cardiovascular disease. Inhibitors of the renin-angiotensin system (RAS) are first-line antihypertensives in patients with CKD, such as diabetic nephropathy, because it has been shown that RAS inhibitors are renoprotective, whether given in early [1, 2] or advanced [3, 4] stages of CKD. Recently, it was reported that RAS inhibitors also suppress the development of diabetic nephropathy when they are administered in the pre-nephropathic stage of diabetes. For example, the angiotensin receptor blocker (ARB) olmesartan prevents the onset of nephropathy in diabetic patients with normoalbuminuria [5]. However, despite the solid evidence showing the renoprotective effects of RAS inhibitors [1–5], RAS inhibitor monotherapy is not believed to be sufficient for CKD associated with hypertension. This is because the target blood pressure (BP) levels critical for suppressing the progression of CKD cannot be achieved by RAS inhibitor monotherapy in the majority of hypertensive patients with CKD. For example, an ARB monotherapy decreases BP to <130/80 mmHg in only 10–20% of hypertensive patients [6]. Thus, second-line antihypertensives are required to manage hypertensive patients with CKD.

The guidelines for the treatment of hypertension recommend dihydropyridine-type calcium channel blockers (CCBs) or thiazide diuretics as candidate antihypertensives that could be combined with RAS inhibitors to treat CKD patients [7–9]. ACCOMPLISH (Avoiding Cardiovascular Events through Combination Therapy in Patients Living with Systolic Hypertension) demonstrated that the cardiovascular risk of the high-risk hypertensive patients is lower if they are treated with benazepril plus amlodipine than if they are treated with benazepril plus hydrochlorothiazide, but the primary endpoints of this study did not include kidney injury [10]. In contrast, GUARD (Gauging Albuminuria Reduction with Lotrel in Diabetic Patients with Hypertension) showed that hydrochlorothiazide plus benazepril decreases the urinary albumin levels in patients with diabetic nephropathy more potently than amlodipine plus benazepril [11]. These observations are consistent with those of REIN (Renoprotection in Patients with Non-diabetic Chronic Renal Disease)-2, which showed that strict BP control due to the addition of felodipine cannot decrease the morbidity of end-stage kidney disease (ESKD) in ramipril-treated non-diabetic patients with proteinuria [12]. An ACCOMPLISH sub-study [13] showed that when benazepril-treated patients (the majority of whom had normal kidney function) were treated with hydrochlorothiazide, the increase in serum creatinine (Cr) were higher than when amlodipine was given; nevertheless, the incidence of ESKD (dialysis and estimated glomerular filtration rate [eGFR] of <15 mL/min/1.73 m^2) in the two groups did not differ. Moreover, the antialbuminuric effect of benazepril plus hydrochlorothiazide was greater in subgroup with CKD than that of benazepril plus amlodipine. Thus, it appears that in combination with RAS inhibitors, thiazide diuretics are superior compared to CCBs in terms of renoprotective effects. However, ACCOMPLISH [10] suggests that CCBs are superior in terms of cardiovascular protective effects. Thus, it is difficult to decide which drugs should be combined with RAS inhibitors to treat CKD patients, especially since these patients often also have cardiovascular disease, which is a major determinant of their life prognosis.

Cilnidipine has been suggested to have sympatholytic effects [14, 15]. Recently, we demonstrated that cilnidipine ameliorates proteinuria in hypertensive patients with CKD whereas the non-sympatholytic CCB amlodipine does not [16]. Supporting this is that the sympathetic nervous system may play an important role in the progression of CKD [17, 18]. Another sympatholytic type of CCB, azelnidipine [19–23], has also been reported to have greater antiproteinuric effects in non-diabetic hypertensive patients with CKD than amlodipine [24]. Moreover, azelnidipine decreases urinary albumin levels in hypertensive patients with diabetic nephropathy more effectively than nifedipine, a non-sympatholytic CCB [25]. Unfortunately, the latter two clinical studies were performed only in a small number of patients.

These observations led us design a large clinical study that addresses the question: does the sympathlytic CCB azelnidipine has a greater antialbuminuric effect in ARB-treated hypertensive patients with diabetic nephropathy than the non-sympatholytic CCB amlodipine? For this study, olmesartan medoxomil was selected to be as the ARB because its antihypertensive effect is potent [6] and it is widely prescribed in both Japan and other countries. This study described here will show one of the best CCBs that can be used in combination with ARB in hypertensive patients with CKD.

Methods

The J-FLAG (Japanese evaluation between FormuLa of Azelnidipine and amlodipine add on olmesartan to Get antialbuminuric effect study) is a prospective, multi-center, open-labeled, randomized trial that is being performed in Japan. It has been registered at the University Hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR) under the trial identification number UMIN000001666. It has been approved by the Institutional Review Boards of the University of Tokyo Clinical Research Center (reference number P2008029-11X) and by the review boards of all the other participating hospitals, and it is being conducted in accordance with the Declaration of Helsinki Principles. Consequently, written informed consent is obtained after patients receive an oral and written explanation of the trial from the attending physician.

Participants

The participants are olmesartan-treated hypertensive patients with diabetic nephropathy. The inclusion criteria are 1) an outpatient systolic BP of ≥130 mmHg and <180 mmHg and/or a diastolic BP of ≥80 mmHg and <110 mmHg, 2) urinary albumin/Cr ≥30 mg/g in spot urine, 3) fasting blood sugar ≥126 mg/dL or treatment with antidiabetic agents, 4) serum Cr ≤2.0 mg/dL, 5) age ≥20 and <80 year-old, and 6) ≥1 month of treatment with olmesartan (10 to 20 mg/day) and no treatment with CCB.

The exclusion criteria are 1) a hypertensive emergency that required an intravenous administration of antihypertensives, 2) nephrotic syndrome (urinary protein ≥3.5 g/day and serum total protein ≤6.0 g/dL [or serum albumin ≤3.0 g/dL]), 3) the administration of contraindication drugs (angiotensin-converting enzyme [ACE] inhibitors, ARBs other than olmesartan, CCBs, adrenocorticosteriods, immunosuppressants, azole antifungal agents [such as itraconazole or miconazole], HIV protease inhibitors [such as ritonavir, saquinavir, or indinavir]) or long-term (≥2 weeks) administration of non-steriod anti-inflammatory drugs (NSAID), 4) a past history showing that CCBs, ARBs, or ACE inhibitors have severe side effects, 5) cerebrovascular disease that occurred within 6 months before registration, 6) sever heart failure (NYHA class ≥III), severe arrhythmia (frequent ventricular or atrial extrasystole, prolonged ventricular tachycardia, atrial tachyrhythmia with severe tachycardia, atrial fibrillation or flutter with severe tachycardia, sick sinus syndrome with severe bradycardia, or atrio-ventricular block with severe bradycardia), myocardial infarction or percutaneous transluminal coronary angioplasty within 6 months before registration, 7) type 1 diabetes or type 2 diabetes required hospitalization due to high hemoglobin A1c levels (≥9.0), extremely high blood glucose levels, or diabetic keto-acidosis, 8) aspartate amino transferase (AST) and alanine transaminase (ALT) levels that are ≥5 times the upper limit of normal, 9) malignancy, and 10) pregnancy, the possibility of pregnancy, or the desire to become pregnant.

Study design

During each initial screening visit, written informed patient consent is obtained, interim registration is performed and all examinations are conducted to evaluate patient eligibility. After confirming patient eligibility, each patient is officially registered and randomly allocated by using a permuted-block design into one of two groups; namely, azelnidipine (started at 8 mg/day, then adjusted to 8–16 mg/day) or amlodipine (started at 2.5 mg/day, then adjusted to 2.5–5 mg/day) in combination with olmesartan (10–20 mg/day) (Fig. 1). The following factors will be used for stratified randomization: 1) urinary albumin/Cr ratio (<300 mg/g, ≥300 and <1,000 mg/g, >1000 mg/g), and 2) systolic BP (<145 mmHg, ≥145 mmHg). Azelnidipine or amlodipine is started 1 month after the observation period commences. The dose of olmesartan is not changed during the treatment period. The target BP is <130/80 mmHg. If azelnidipine or amlodipine fails to reduce BP to the target level, additional antihypertensive drugs (other than a RAS inhibitor or a CCB) are administered. The treatment period is 12 months.

Outcome measures

The primary endpoint is changes in the urinary albumin/Cr ratio in spot urine after 12 months of treatment relative to the ratio at the pretreatment period (average of two measured values) to 12 months of treatment.

The secondary outcomes are 1) changes in the urinary albumin/Cr ratio at each treatment period relative to the pretreatment period value, 2) urinary protein/Cr ratio, 3) urinary liver-type free fatty acid-binding protein (L-FABP)/Cr ratio, 4) urinary 8-hydroxydeoxyguanosine (8-OHdG)/Cr ratio, 5) office BP in outpatient clinic, 6) pulse rate, 7) eGFR calculated using the Modified Diet in Renal Disease (MDRD) formula that was modified by the Japanese Society of Nephrology [26], and 8) cerebro-cardiovascular events, which include cerebro-cardiovascular death (fatal myocardial infarction, fatal heart failure, sudden death, fatal stroke, and other cardiovascular deaths) and hospitalization due to cerebro-cardiovascular disease (nonfatal myocardial infarction, angina pectoris, heart failure, cerebral bleeding, cerebral infarction, and transient cerebral ischemic attack).

Laboratory tests of urine are performed at a central laboratory (SRL Medisearch Inc., Tokyo, Japan). The urinary albumin level is measured by a turbidimetric

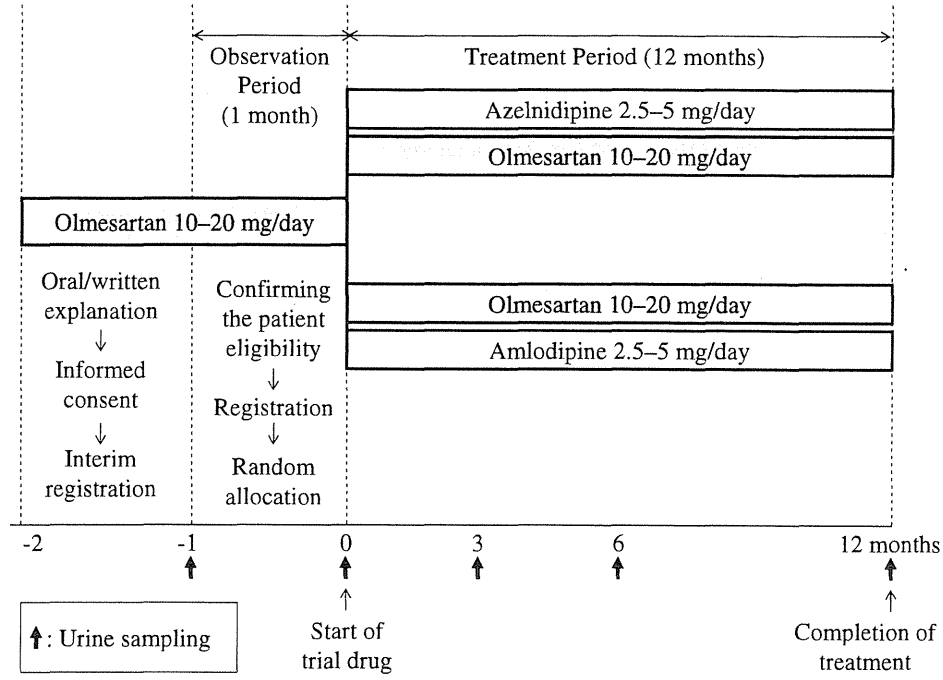

Fig. 1 Design of the J-FLAG (Japanese evaluation between FormuLa of Azelnidipine and amlodipine add on olmesartan to Get antialbuminuric effect study) trial

immunoassay (AutoWako Microalbumin, Wako Pure Chemical Industries, Ltd., Osaka), and the Cr level is measured by an Enzymatic Colorimetric Assay (Pure Auto S CRE-L, Sekisui Medical Co., Ltd., Tokyo). Both assays employ the 7700 auto-analyzer (Hitachi High-Technologies Corp., Tokyo). Urinary L-FABP is examined by an enzyme-linked immunosorbent assay (ELISA: Human L-FABP Assay Kit-IBL: Immuno-Biological Laboratories Co. Ltd., Takasaki). Urinary 8-OHdG is also measured by an ELISA (New 8-OHdG Check ELISA, Japan Institute for the Control of Aging, Nikken Seil Co., Ltd., Fukuroi).

Sample size determination

Based on the previous results of studies on azelnidipine [24, 25, 27], it was assumed that azelnidipine and amlodipine will differ in terms of logarithmically-transformed changes in the urinary albumin/Cr ratio after 12 months of the treatment by 0.15 and with a standard deviation of 0.37. To detect a difference in the primary endpoint between the two arms with an alpha error of 5% and a power of 80%, 194 patients will be required. Assuming a 20% loss to follow-up, 250 patients (125 per arm) were planned to be enrolled.

Statistical considerations

For the efficacy endpoint, the primary analysis will be carried out on the intent-to-treat (ITT) population (i.e. all randomized patients, regardless of patient compliance, actual administration of the trial drug, or premature trial drug discontinuation), not including those patients who are deemed ineligible (see eligibility criteria above) or who never take the trial drug. In terms of the safety endpoint, all patients who take the trial drug at least once will be analyzed.

The azelnidipine and amlodipine groups will be compared in terms of the logarithmically-transformed change in urinary albumin/Cr ratio after 12 months of treatment relative to the pretreatment value; for this, analysis of covariance will be employed. To include missing values in the comparison of the effects between azelnidipine and amlodipine, the data will be analyzed by using mixed model separately. The two-sided significance level is 5%. The absolute values and changes over time of the secondary outcome measures will also be analyzed. To evaluate trial drug safety, the two groups will be also compared in terms of the frequency of adverse events and the rate of dropout due to adverse events.

Management of the study

The organization and members of each committee of the J-FLAG trial are shown in the Appendix. The Principal Study Coordinator and the Steering Committee oversee and are responsible for conducting the trial, regarding protocol changes, and premature study termination. The Steering Committee is blinded to the treatment assignments and takes responsibility for publications arising from the trial. The Protocol Committee is responsible for the study design and protocol development and their changes. The Executive Study Coordinator and Coordinating Committee are responsible for the implementation of the trial,

fund management, data management, statistical analysis, and general affairs. The Data Monitoring and Safety Committee assesses safety and endpoints, evaluates adverse events, oversees patient welfare, reviews trial data at specified intervals, and makes recommendation to the Steering Committee if any problems arise (e.g. serious adverse events). This committee does not include investigators in the study.

Discussion

The J-FLAG trial asks whether the sympatholytic CCB azelnidipine has a more potent antialbuminuric effect in olmesartan-treated hypertensive patients with diabetic nephropathy than the non-sympatholytic CCB amlodipine. Generally, glomerular hypertension plays an important role in the onset and progression of CKD. For example, a recent meta-analysis has shown that diabetic patients who have normal renal function at diagnosis but exhibit accelerated progression of kidney injury in the future have an increased baseline glomerular filtration rate, which is probably due to glomerular hyperfiltration [28]. L-type CCBs, which are commonly used as antihypertensives, have a glomerular pressure-increasing effect because they induce vasodilation of the glomerular afferent but not efferent arteries [29]. This glomerular hemodynamic change may cancel or attenuate the glomerular pressure reduction induced by the hypotensive action of CCBs. In contrast, the alpha$_1$ blocker terazosin decreases glomerular pressure by inducing the vasodilation of both afferent and efferent arteries in spontaneously hypertensive rats [30]. Thus, some CCBs such as azelnidipine, which have a sympathlytic action, may be beneficial for patients with CKD. Indeed, a videomicroscope study has shown that the sympathlytic CCB azelnidipine increases the diameter of both afferent and efferent arteries in angiotensin II-infused rats and that this is renoprotective effects [31]. In addition, our previous study [16] showed that another sympatholytic CCB decreased urinary protein/Cr ratio more potently than amlodipine. Moreover, two small clinical studies revealed that azelnidipine has greater antiproteinuric or antialbuminuric effects than the non-sympatholytic CCB amlodipine or nifedipine [24, 25]. Thus, to clarify whether a sympatholytic CCB inhibits the progression of kidney injury, the J-FLAG trial was designed to test the renoprotective effect of azelnidipine.

Although CCBs and diuretics are recommended to add as second-choice antihypertensives that could be added to RAS inhibitor treatment for patients with diabetic nephropathy and hypertension, each combination, as indicated in the Introduction, is good for one clinical aspect but not the other. Thus, while the CCBs plus RAS inhibitors combination appears to ameliorate cardiovascular prognosis better than the diuretics and RAS inhibitors combination, it is less effective in terms of preventing renal injury. Since cardiovascular disease is a major cause of death in CKD patients, the most desirable antihypertensives for the treatment of CKD are those that effectively suppress both cardiovascular disease and renal injury. This objective may be met by combining RAS inhibitors with sympatholytic CCBs. Supporting this is that azelnidipine combined with olmesartan has a more potent vasoprotective effect in mice with cuff-placement vascular remodeling than hydrochlorothiazide combined with olmesartan [32]. In addition, the J-CORE (Japan-Combined Treatment with Olmesartan and a Calcium Channel Blocker versus Olmesartan and Diuretics Randomized Efficacy) trial demonstrated that azelnidipine plus olmesartan ameliorates aortic pulse wave velocity and augmentation index in hypertensive patients better than hydrochlorothiazide plus olmesartan [33]. Thus, azelnidipine in combination with olmesartan may have cardiovasdcular protective effects. This means that azelnidipine in combination with a RAS inhibitor is expected to suppress the progression of CKD as well as cardiovascular disease. This combination may be one of the best choices for the treatment of CKD patients.

In Japan, azelnidipine is used in combination drug with olmesartan [22, 23]. This is different from other ARB-CCB combinations that are commonly used around the world, which generally employ amlodipine. The J-FLAG trial will clarify whether the sympatholytic CCB azelnidipine is a beneficial second-line choices for treating RAS inhibitor-treated hypertensive patients with CKD, such as diabetic nephropathy. It may indicate that the olmesartan-azelnidipine combination is recommended for CKD patients who frequently have a health-threatening cardiovascular disease.

Source of Funding The J-FLAG trial was funded by the Waksman Foundation of Japan Inc.

Appendix

The J-FLAG (Japanese evaluation between FormuLa of Azelnidipine and amlodipine add on olmesartan to Get antialbuminuric effect study) group

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Executive Study Coordinator

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Sadayoshi Ito, Division of Nephrology, Endocrinology, and Vascular Medicine, Department of Internal Medicine, Tohoku University Graduate School of Medicine

Kenjiro Kimura, Department of Nephrology and Hypertension, St. Marianna University School of Medicine

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Naoki Kashihara, Division of Nephrology, Department of Internal Medicine, Kawasaki Medical School

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Hiroaki Matsuoka, Utsunomiya Chuoh Hospital (Chairman)

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Masaomi Nangaku, Department of Nephrology and Endocrinology, Faculty of Medicine, University of Tokyo

Tatsuo Shimosawa, Department of Clinical Laboratory, Faculty of Medicine, University of Tokyo

Advisory Board for Statistical Analysis

Junji Kishimoto, Center for Clinical and Translational Research, Kyushu University Hospital

Data Monitoring and Safety Committee

Tanenao Eto, Miyazaki Prefectural Health Foundation (Chairman)

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Renovascular Protective Effects of Erythropoietin in Patients with Chronic Kidney Disease

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Abstract

Background/Aims Erythropoietin (EPO) has been widely used for the treatment of anemia in chronic kidney disease (CKD). A growing body of evidence indicates that the therapeutic benefits of EPO could extend beyond the improvement of anemia. The aim of the present study was to determine whether EPO affects renovascular and oxidative stress biomarkers in pre-dialysis CKD patients with anemia.

Methods The study was a single-arm prospective study. Fifteen CKD patients (9 males and 6 females, mean age 63 years) with anemia (mean Hb: 8.1 g/dL) were treated with recombinant human EPO; 12,000 U administered subcutaneously once every 2 weeks. Various parameters were measured before and 6 months after treatment. These included serum hemoglobin (Hb), creatinine, estimated glomerular filtration rate (eGFR), proteinuria, urinary liver-type fatty acid binding protein (L-FABP - a biomarker of renal injury), urinary 8-hydroxydeoxyguanosine (8-OHdG - a marker of oxidative stress), serum asymmetrical dimethylarginine (ADMA), carotid artery intima-media thickness (IMT) and brachial-ankle pulse wave velocity (baPWV) as vascular markers and plasma brain natriuretic peptide (BNP) levels and left ventricular ejection fraction (LVEF) as cardiac function markers and cardio-thoracic ratio (CTR) and inferior vena cava dimension (IVCS) as extra fluid retention markers.

Results After 6 months, serum Hb was significantly increased ($p<0.001$) and urinary levels of protein, L-FABP and 8-OHdG, carotid IMT, baPWV, plasma BNP and serum ADMA levels were significantly decreased ($p<0.001$). Serum creatinine, eGFR, LVEF, CTR and IVCS showed little difference throughout the experimental period.

Conclusion These data suggest that recombinant human EPO may ameliorate renal injury, oxidative stress and progression of atherosclerosis in addition to improving anemia in CKD patients.

Key words: erythropoietin, CKD, drug

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Introduction

Erythropoietin (EPO), the principal hematopoietic hormone produced by the kidney and the liver, regulates mammalian erythropoiesis and exhibits diverse cellular effects in non-hematopoietic tissues (1). EPO has been shown to significantly protect multiple organs in both acute and chronic diseases (1, 2). Urinary liver-type fatty acid binding protein (L-FABP) is a useful biomarker that reflects renal tubulointerstitial injury and can be used to monitor both hemodynamic and drug responses in animal models and patients with chronic kidney disease (CKD) (3, 4). Urinary 8-hydroxydeoxyguanosine (OHdG) is a marker of oxidative stress and is associated with the progression of CKD (5, 6). Kasap et al (7) reported that EPO had no significant protective effect upon renal function or chronic fibrosis in animal models but it did reduce tubular changes, apoptosis and oxidative stress. In addition, Bi et al (8) reported that EPO can protect tubular cells from various injurious stimuli both *in*

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vitro and *in vivo*. However, there is little known regarding the effect of EPO on urinary L-FABP and urinary 8-OHdG levels in CKD patients.

Carotid artery intima-media thickness (IMT) and brachial-ankle pulse wave velocity (baPWV) have been extensively utilized as markers of vascular function in CKD patients (9, 10). Asymmetrical dimethylarginine (ADMA) is an endogenous inhibitor of endothelial nitric oxide synthase (eNOS) and has been reported to be a novel marker for the progression of CKD with ADMA accumulation triggering peritubular capillary loss that contributes to tubulointerstitial ischemia and fibrosis (11). In addition, ADMA is strongly associated with carotid artery IMT (12). However, there is also little known regarding the effect of EPO on IMT, PWV and ADMA in CKD patients.

Brain natriuretic peptide (BNP) is a biomarker of cardiac function with cardiac dysfunction being common in CKD patients (13). CKD patients with left ventricular diastolic dysfunction have significantly higher BNP levels than patients with normal cardiac function (14). However, little is known regarding the effect of EPO on BNP levels in CKD patients. Therefore the present study was conducted to examine the effect of treatment with recombinant human EPO on cardiac and renovascular function and oxidative stress in pre-dialysis CKD patients with anemia.

Materials and Methods

Patients

The study was a single-arm prospective study that included 15 non-diabetic CKD patients with anemia (9 males and 6 females, age 63±6 years, serum creatinine level 3.92± 1.21 mg/dL, eGFR 13.26±4.84 ml/min and hemoglobin [Hb]: 8.1±0.5 g/dL). None of the patients had diabetes, systemic inflammatory disorders including vasculitis, collagen disease, liver disease or malignancy. CKD was diagnosed by renal biopsy with diagnoses including IgA nephropathy (n= 9), non-IgA proliferative glomerulonephritis (n=3), nephrosclerosis (n=2) and focal glomerular sclerosis (n=1). Anemia was defined as a hemoglobin level of less than 10 g/dL upon entry into the study. None of the patients had received previous treatment with recombinant human EPO. Concomitant drugs prescribed were anti-hypertensive drugs (12 patients), statins (3 patients), the carbonaceous oral adsorbent AST-120 (8 patients), anti-platelet drugs (6 patients) and prednisolone (2 patients). AST-120 adsorbs various uremic retention solutes in the gastrointestinal system (15). These drugs were not changed during the study period. All patients received subcutaneous injection of recombinant human EPO (epoetin beta) (Chugai Phamaceutical Co., Ltd, Tokyo, Japan) at an initial dose of 12,000 U every 2 weeks with the dose subsequently adjusted to achieve a hemoglobin level of 12 g/dL for 6 months. During the study period, the patients were also given oral iron supplements to maintain their serum ferritin level and transferrin saturation index at greater than 150 μl/L and 20%, respectively (16). The study protocol was approved by the local ethical committee of Shinmatsudo Central General Hospital and informed consent was obtained from all study participants. The ethical committee did not approve a placebo controlled study because of the risk it would pose to the patient; this study was therefore performed as a single-arm study. The study complied with the principles of the Helsinki Declaration.

Measurements

Blood pressure (BP) was measured in the sitting position after 2 minutes of rest using an upright standard sphygmomanometer. Renal function was evaluated by serum creatinine level and estimated glomerular filtration rate (eGFR), based upon the Modification of Diet in Renal Disease (MDRD) equation modified for the Japanese population (17). Total urinary protein excretion was determined with the pyrogallol red method (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The urinary L-FABP levels were measured by a specific enzyme-linked immunosorbent assay (ELISA) (CIMIC Co., Ltd., Tokyo, Japan) as described previously (18). The urinary L-FABP value was expressed as a ratio of the urinary creatinine concentration (18). Urinary 8-OHdG levels were measured by specific ELISA as previously described (NIKKEN SEIL Co., Ltd., Shizuoka, Japan) (19). Serum ADMA was measured by high-performance liquid chromatography (20, 21). BaPWV, as measured using a sphygmomanometer and sphygmograph (COLIN Medical Technology, Tokyo, Japan) (22), was expressed in centimeters per second. Carotid artery IMT was measured using high-resolution B-mode ultrasound examination with a 7.5 MHz mechanical sector transducer, the Aloka SSP-2000 (Hitachi Aloka Medical, Ltd., Tokyo, Japan) (22). Sonographic data were obtained by an experienced technician with no knowledge of the patient's clinical data. LVEF was calculated by a Sonos 4500 echographic tomography (Philips Electronics Japan, Tokyo, Japan). Plasma BNP level was determined by radioimmunoassay (Shionogi Pharmaceutical Co., Ltd., Osaka, Japan) (23, 24). In addition, we measured the cardio-thoracic ratio (CTR) and inferior vena cava dimension (IVCD). Table 1 shows Baseline Characteristics.

Statistics

Data are expressed as mean ± SD. Differences were analyzed by paired Student's t test. A p-value of less than 0.05 was considered significant. All statistical analyses were performed using the SAS system (SAS Institute, Cary, NC, USA).

Results

All patients completed the study protocol and there were no adverse effects of EPO treatment during the study period. No patient received a blood transfusion during the study period. At 6 months after treatment with EPO, the Hb level in-

Intern Med 50: 1929-1934, 2011 DOI: 10.2169/internalmedicine.50.5145

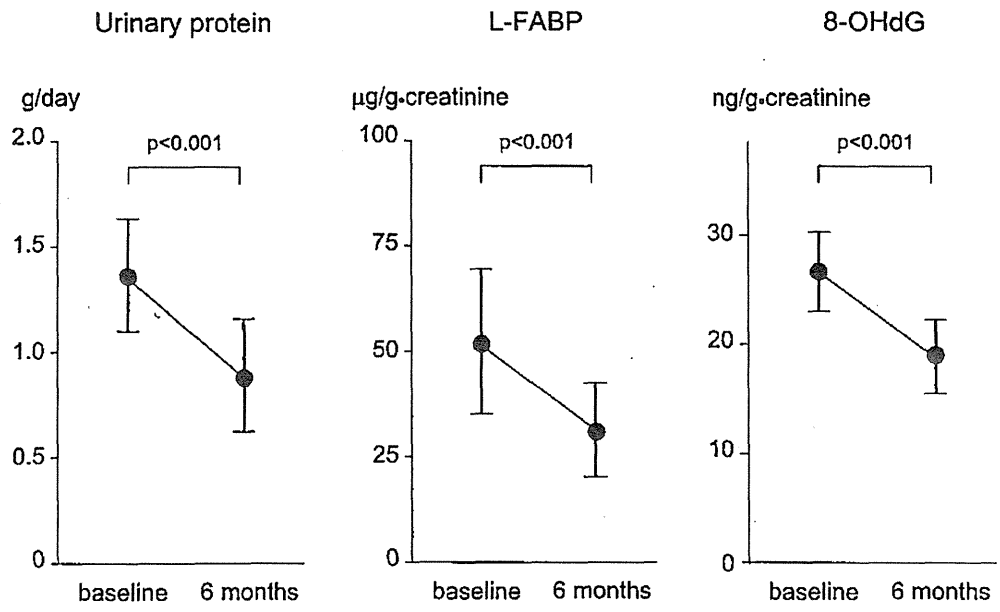

Figure 1. Changes in urinary protein excretion, urinary L-FABP level and urinary 8-OHdG levels before and after 6 months of EPO treatment.

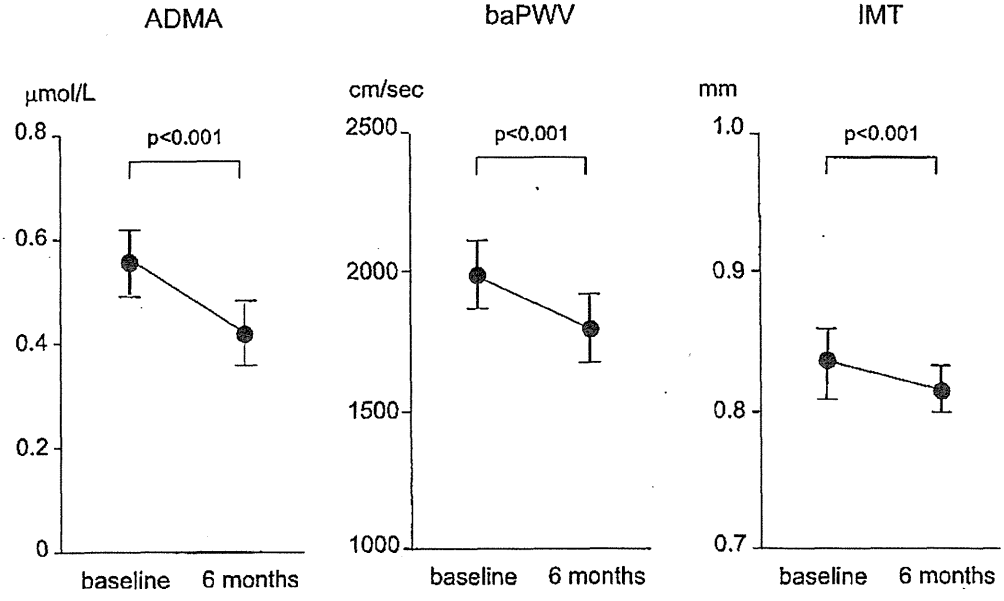

Figure 2. Changes in serum ADMA level, baPWV and IMT before and after 6 months of EPO treatment.

creased from the baseline value of 8.1±0.5 g/dL to 11.4±0.7 g/dL ($p<0.001$). There was no change in the serum creatinine levels, eGFR or BP. There was a significant reduction in urinary protein excretion (1.39±0.32 to 0.89±0.22 g/day, $p<0.001$), urinary L-FABP levels (53.3±18.7 to 32.2±11.6 μg/g creatinine, $p<0.001$) and urinary 8-OHdG levels (26.6±2.8 to 19.4±4.2 ng/mg creatinine, $p<0.001$) (Fig. 1). The LVEF did not change significantly (62±6 to 64±10%) whilst serum BNP levels decreased (77±20 to 48±13 pg/mL, $p<0.001$). The CTR and IVCD did not change significantly (CTR: 46.5±3.6% to 46.2±3.8%, IVCD: 12.5±2.2 mm to 12.7±2.4 mm). The serum ADMA levels (0.561±0.088 to 0.425±0.066 μmol/L, $p<0.001$), baPWV (1955±218 to 1793±184 cm/s, $p<0.001$) and carotid artery IMT (0.834±0.052 to 0.826±0.038 mm, $p<0.001$) were significantly decreased during the study (Fig. 2).

Discussion

In the present study, we demonstrated that 6 months of

Intern Med 50: 1929-1934, 2011 DOI: 10.2169/internalmedicine.50.5145

Table 1. Baseline Characteristics

Patient number (n)	15
Age (years)	63±8
Sex (male/female)	9/6
Serum creatinine (mg/dL)	3.92±1.21
eGFR (mL/min)	13.26±4.84
Hb (g/dL)	8.1±0.5
Proteinuria (g/day)	1.39±0.32
Urinary L-FABP (μg/g creatinine)	53.3±18.7
Ejection fraction (%)	62±6
BNP (pg/mL)	77±20
Urinary 8-OHdG (ng/mg creatinine)	26.6±2.8
ADMA (μmol/L)	0.56±0.09
CTR (%)	46.5±3.6
baPWV (cm/s)	1955±218
carotid artery IMT (mm)	0.834±0.052
IVCD (mm)	12.5±2.2
SBP (mmHg)	128±5
DBP (mmHg)	76±4
Primary disease (n)	
IgA nephropathy	9
Nephrosclerosis	3
Non-IgA PGN	2
FGS	1
Concomitant drugs (n)	
Anti-hypertension	
ARB	10
ACEI	3
Ca antagonist	6
Alpha antagonist	3
Diuretics	5
Statin	3
AST-120	8
Anti-platelet	6
Prednisolone	2
Iron	15

eGFR = estimated glomerular filtration rate, L-FABP = liver-type fatty acid binding protein, 8-OHdG = 8-hydroxydeoxyguanosin, BNP = brain natriuretic peptide, ADMA =asymmetric dimethylarginine, baPWV = brachial ankle pulse wave velocity, IMT = intima-media thickness, SBP = systolic blood pressure, DBP = diastolic blood pressure, PGN = proliferative glomerulonephritis, FGS = focal glomerulosclerosis, ARB = angiotensin II receptor blocker, ACEI = angiotensin converting enzyme inhibitor, CTR=cardio-thracic ratio, IVCD=inferior vena cava dimention

therapy with recombinant human EPO significantly reduced urinary protein excretion and the urinary L-FABP level in pre-dialysis CKD patients with anemia, thereby suggesting that EPO exerts renoprotective effects. The levels of urinary 8-OHdG, plasma BNP and serum ADMA were also reduced after 6 months of EPO treatment, suggesting that EPO also exerts an anti-oxidative and cardioprotective effect and improves NO bioavailability. In addition, the reduction of carotid artery IMT and baPWV suggests a beneficial vascular protective effect of EPO.

A growing body of experimental evidence indicates that there are therapeutic benefits of EPO beyond the correction of anemia. Several articles have recently reported the tissue protective, non-hematological effects of EPO that prevent or limit tissue damage in several organs including the kidney (25). EPO is thus a very promising cytoprotective agent to promote cell survival in both acute and chronic conditions. Toyeux-Faure (1) reviewed the mechanisms underlying the cytoprotective effect of EPO including the role of the EPO receptor and activation of cellular signaling pathways. EPO ameliorates tubulointerstitial injury in the model of unilateral ureteral obstruction by inhibiting inflammation, interstitial fibrosis and tubular apoptosis (26). Bone marrow stromal cells (BMSC) exert a renoprotective effect in tubular injury via the secretion of factors that reduce apoptosis and increase proliferation of tubular epithelial cells (8). EPO receptors are expressed on the surface of BMSCs and EPO protects BMSCs from cell death induced by serum deprivation and stimulates BMSC proliferation *in vitro* (8). EPO diminishes renal injury associated with cisplatin administration (8) and protects primary mouse tubular epithelial cells from necrotic ischemic injury via the JAK2/Y343/STAT5 signaling pathway (27). In the present study, EPO treatment reduced the urinary excretion of the biomarker L-FABP lending clinical support to these experimental data. In addition, EPO promotes cytoprotection by anti-oxidative mechanisms such as inhibiting heme oxygenase-1 and glutathione peroxidase (28). In the present study, we clinically confirmed the anti-oxidative effects of EPO by demonstrating that it significantly reduced urinary 8-OHdG levels.

ADMA is a naturally occurring amino acid found in plasma and various tissues. The blood level of ADMA is re-

Intern Med 50: 1929-1934, 2011 DOI: 10.2169/internalmedicine.50.5145

ported to be associated with cardiovascular risk factors such as hypertension, diabetes, dyslipidemia and CKD and is a strong predictor of cardiovascular disease and progression of CKD (29). ADMA injures the glomerular filtration barrier (30) and Ueda et al (11) reported that ADMA may be involved in glomerular capillary loss and sclerosis thereby contributing to the progression of CKD. In the present study, we recognized that the degree of change in proteinuria was correlated with that in serum ADMA levels ($r2=0.817$, $p<0.001$, data not shown). This may suggest that EPO reduced proteinuria, possibly as a result of reducing blood ADMA levels. High ADMA levels are associated with increased carotid artery IMT which is a validated surrogate marker for atherosclerosis (31). Carotid artery IMT was significantly higher in subjects with early stage CKD and the greater prevalence of cardiovascular disease risk factors in patients with CKD accounted for the higher carotid artery IMT (32). Pawiak et al (33) reported that IMT values were significantly decreased in dialysis patients who received EPO for more than 12 months compared to untreated patients. The mechanisms responsible for the high baPWV seem to be associated with increased left ventricular systolic pressure, decreased coronary flow due to decreased diastolic blood pressure as well as direct effects on the progression of atherosclerosis and left ventricular dysfunction (34). The result of the present study indicates that EPO reduced IMT and baPWV suggesting an anti-atherosclerotic effect in CKD patients.

The diagnosis or exclusion of heart failure is important in CKD patients given the high prevalence of left ventricular hypertrophy and left ventricular systolic dysfunction in this population (35). In addition, CKD patients exhibit a high prevalence of diastolic dysfunction (14). Plasma BNP may be an appropriate biomarker to screen for both systolic and diastolic cardiac dysfunction in CKD (13). Similar to observations in patients with normal renal function, the plasma BNP level has been reported to be a prognostic indicator of cardiovascular disease development and cardiovascular death in CKD patients. There is some possibility that the reduction of BNP results from a reduction of extracellular fluid volume rather than an improvement in diastolic function. The CTR and IVCD as indicators of extracellular fluid volume did not change significantly in the present study. The results of the present study indicating that EPO reduced plasma BNP levels without changing the LVEF, CTR and IVCD in CKD patients suggest that the major effect of EPO may predominantly be an improvement of diastolic function.

Limitations

There are several limitations to this study. This was a preliminary single-arm study that was designed for only 6 months with a small number of subjects. A multi-center, large scale, comparative study over a longer period would be needed in the future. In addition, we used some concomitant drugs such as ARB, statin and AST-120, which may potentially affect the changes in each marker observed in this study. Many reports suggest that EPO has a cytoprotective effect, however, in the present study, we found little direct evidence of a cytoprotective effect of EPO.

In conclusion, recombinant human EPO may be effective in ameliorating renal injury, atherosclerosis and oxidative stress in pre-dialysis CKD patients with anemia.

The authors state that they have no Conflict of Interest (COI).

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Potential Benefit of Statin Therapy for Dyslipidemia with Chronic Kidney Disease: Fluvastatin Renal Evaluation Trial (FRET)

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Abstract

Background Dyslipidemia is a common complication of chronic kidney disease (CKD) and contributes to cardiovascular morbidity and mortality of CKD patients.

Aim The aim of the present study was to determine whether fluvastatin, which is mostly characterized by its pleiotropic anti-oxidant effects, has renoprotective effects in dyslipidemic patients with CKD.

Methods In 43 dyslipidemic patients with CKD taking fluvastatin 10 mg/day, 20 mg/day or 30 mg/day, renal functions as well as lipid profiles were assessed.

Results After 3 months of treatment with fluvastatin, LDL-cholesterol level significantly decreased. Serum creatinine level, estimated glomerular filtration rate (eGFR), urinary albumin excretion (UAE), urinary liver-type fatty acid binding protein (L-FABP) level and urinary 8-hydroxydeoxyguanosine (8-OHdG) level did not change in overall patients. However, in patients with microalbuminuria (baseline UAE≥30 mg/g·creatinine; n=23), the UAE significantly decreased [2.43±0.67 to 1.98±0.80 log(mg/g·creatinine), p=0.01]. In patients with high L-FABP group (baseline L-FABP≥11 μg/g·creatinine; n=18), the urinary L-FABP level was significantly decreased (1.52±0.45 to 1.26±0.43 μg/g·creatinine, p<0.01). In the limited 23 patients with microalbuminuria, the L-FABP level was significantly decreased [1.20±0.62 to 1.03±0.49 log(μg/g·creatinine), p=0.042], although the LDL-cholesterol level (139±28 to 129±23 mg/dL, p=0.08) only showed a tendency to decrease. The 8-OHdG level also was significantly decreased (13.6±9.6 to 9.8±3.8 ng/g·creatinine, p=0.043). In the overall patients, changes in the values for UAE and urinary L-FABP were not correlated with the changes in LDL-levels.

Conclusion Fluvastatin reduces both UAE and the urinary L-FABP level, and thus, has renoprotective effects, independent of its lipid lowering effects in dyslipidemic patients with CKD.

Key words: statin, chronic kidney disease, oxidative stress, urinary albumin excretion, L-FABP

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Introduction

Chronic kidney disease (CKD) in the community is associated with a significant burden of cardiovascular disease risk factors (1). The prevalence of dyslipidemia in CKD patients is much higher than in the general population whilst elevated cholesterol and triglyceride levels are associated with more rapid deterioration of kidney function (2). Thus, CKD is a "high risk" category for cardiovascular events and aggressive therapeutic intervention should be initiated to reduce the risk (3). Recently, Sandhu et al reported that ther-

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Table 1. Patient Characteristics (n=43)

Age; years	75±9
Sex; male/female	21/22
Hypertension; n (%)	33 (76)
Diabetes; n (%)	14 (33)
Smoking; n (%)	5 (12)
Cadriovascular diseases; n (%)	9 (21)
Basal diseases of CKD	
Hypertensive nephropathy; n (%)	27 (63%)
Diabetic nephropathy; n (%)	8 (19%)
Either or both of HN and/or DN ; n (%)	6 (14%)
Chronic glomerulonephritis; n (%)	2 (4%)
Fluvastatin dose	
10 mg/day; n (%)	7 (16)
20 mg/day; n (%)	31 (72)
30 mg/day; n (%)	5 (12)
ACEI or ARB; n (%)	30 (70)
Ca channel blocker; n (%)	26 (60)
Anti-diabetic drugs; n (%)	11 (26)
Aspirin; n (%)	4 (9)

HN=hypertensive nephropathy, DN=diabetic nephropathy, ACEI=angiotensin converting enzyme inhibitor, ARB=angiotensin receptor blocker

apy with cholesterol lowering drugs, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, i.e., statins resulted in a modest reduction in proteinuria and protected renal function in a meta-analysis comprising 39,704 participants (4).

Among the various statins, fluvastatin is primarily characterized by its pleiotropic anti-oxidant effects (5, 6). Since oxidative stress produces renal glomerular injury and tubulointerstitial damage (7), we hypothesize that fluvastatin may have specific beneficial effects to improve renal function. To test this hypothesis, we designed a single arm multi-center study, the Fluvastatin Renal Evaluation Trial (FRET), to assess the effects of fluvastatin on renal function in dyslipidemic patients with CKD.

Methods

For the FRET trial, patients with dyslipidemia along with CKD over the age of 20 years old were recruited from 8 practitioners belonging to Saga Medical Association. Dyslipidemia and CKD were defined based upon the criteria of the Japanese Atherosclerosis Society and the Japanese Society of Nephrology, respectively. Patients who had cardiac, liver, gastrointestinal, or collagen disease, malignancy, or a history of previously receiving any lipid lowering drugs were excluded. All of the patients were prescribed 10 mg, 20 mg or 30 mg fluvastatin. The dose of fluvastatin was dependent upon the judgment of each attending physician. We monitored blood pressure, lipid profiles such as serum low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol and triglyceride levels, and renal function markers such as serum creatinine level, urinary albumin excretion (UAE) and urinary liver-type fatty acid binding protein (L-FABP) level prior to the fluvastatin prescription and 3 months after the fluvastatin treatment. Estimated glomerular filtration rate (eGFR) was calculated based on the Japanese Society of Nephrology CKD Practice Guide. In addition, urinary 8-hydroxydeoxyguanosine (8-OHdG) was simultaneously measured as an oxidative stress marker. L-FABP levels were measured by a specific ELISA method as previously described (8-10). 8-OHdG levels were measured by a specific ELISA kit as previously described using a highly sensitive monoclonal antibody (8-OHdG Check, Nikken Foods, Fukuroi, Shizuoka, Japan) (11).

Normality of the distribution of variables was assessed using Kolmogorov-Smirnov test with Lilliefors' correlation. Since values of UAE and L-FABP were non-parametric, the values were transformed into logarithmic values. Then the data were expressed as mean±SD. The changes in the values after 3 months treatment of fluvastatin were assessed using paired t test. Correlations were assessed using simple linear regression. The P value less than 0.05 was considered to be statistically significant.

Results

A total of 43 patients (21 males and 22 females; 75±9 years) were eligible for the FRET trial. Patient characteristics are shown in Table 1. Complications with cardiovascular diseases were seen in 9 patients (21%) (ischemic heart disease in 3; 7%, arrhythmia in 5; 12%, hypertensive heart disease in 1; 2% and cerebrovascular disease in 3; 7%). Basal diseases of CKD were hypertensive nephropathy in 27 (63%), diabetic nephropathy in 8 (19%), either or both hy-

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Table 2. Changes in the Measurements after 3 Months' Treatment with Fluvastatin

	Baseline	3 months	p value
Systolic blood pressure; mmHg	137±15	135±13	0.0792
LDL-cholesterol; mg/dL	131±30	119±22	0.0097
HDL-cholesterol; mg/dL	50±11	48±12	0.1521
Triglyceride; mg/dL	178±90	160±89	0.0950
Fasting blood glucose; mg/dL	110±30	114±30	0.2696
HbA_1C; %	5.9±1.1	5.9±1.0	0.2157
Creatinine; mg/dL	1.16±0.48	1.18±0.54	0.3419
eGFR; ml/min/1.73m^2	40±14	40±14	0.6800
UAE (n=36); mg/g·creatinine	72.2 [10.0, 355.3]	49.4 [12.5, 282.3]	0.0937
log(mg/g·creatinine)	1.84±0.84	1.75±0.77	0.1662
L-FABP (n=36);mg/g·creatinine	10.5 [4.0, 26.3]	7.4 [4.0, 17.7]	0.1447
log(mg/g·creatinine)	1.07±0.54	0.98±0.43	0.1059
8-OHdG (n=36); ng/mg·creatinine	12.7±8.0	10.9±4.1	0.1705

Data are expressed as mean ± standard deviation or median value and interquartile range. LDL=low density lipoprotein, HDL=high density lipoprotein, HbA1C=hemoglobin A1C, eGFR=estimated glomerular filtration rate, ACEI=angiotensin converting enzyme inhibitor, UAE=urinary albumin excretion, L-FABP=liver type fatty acid binding protein, 8-OHdG=8-hydroxy deoxiguanosine

pertensive nephropathy and/or diabetic nephropathy in 6 (14%) and chronic glomerulonephritis in 2 patients (4%). The dose of fluvastatin was 10 mg/day in 7 (16%), 20 mg/day in 31 (72%) and 30 mg/day in 5 patients (12%). Angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) was given in 30 patients (70%). Although anti-diabetic drugs were given in 11 patients (26%), no patient received thiazolidinediones such as pioglitazone.

Table 2 shows the comparison of measured variables in the baseline before fluvastatin administration and those after 3 months of treatment with fluvastatin in overall patients. After the 3 months of treatment, the LDL-cholesterol level was significantly decreased (131±30 to 119±22 mg/dL, $p<0.01$). However, the levels of HDL-cholesterol and triglyceride did not change. Concerning renal function, the serum creatinine level and eGFR did not change significantly. The UAE, urinary L-FABP level and urinary 8-OHdG level also did not change in 36 patients, in whom these markers could be measured at both baseline and at 3 months after treatment. If these patients are divided into patients with microalbuminuria (baseline UAE≥30 mg/g·creatinine; n=23) and patients without microalbuminuria (baseline UAE<30 mg/g·creatinine; n=13), based on the cut-off value of 30 mg/g·creatinine according to guideline of Japanese Society of Hypertension, the UAE value was significantly decreased in the patients with microalbminuria [2.43±0.67 to 1.98±0.80 log(mg/g·creatinine), p=0.01], although the value did not change in the patients without microalbuminuria [0.96±0.29 to 1.08±0.24 log(mg/g·creatinine)] (Fig. 1). If these patients are divided into two subgroups, a low L-FABP group (baseline L-FABP <11 μg/g·creatinine; n=18) and a high L-FABP group (baseline L-FABP≥11 μg/g·creatinine; n=18), based on the cut-off value of 11 μg/g·creatinine as the median value, the L-FABP level was significantly decreased in the high L-FABP group [1.52±0.45 to 1.26±0.43 log(μg/g·creatinine), $p<0.01$], although the level did not change in the low L-FABP group [0.64±0.11 to 0.72±0.27 log(μg/g·creatinine)] (Fig. 2). In the limited 23 patients with microalbuminuria, the L-FABP level was also significantly decreased [1.20±0.62 to 1.03±0.49 log(μg/g·creatinine), p=0.042], although LDL-cholesterol level (139±28 to 129±23 mg/dL, p=0.08) showed only a tendency to decrease. In these limited patients, the 8-OHdG level was also significantly decreased (13.6±9.6 to 9.8±3.8 ng/g·creatinine, p=0.043) (Fig. 3).

In overall patients, the changes in the values (baseline values minus values after 3 months of treatment) for UAE and urinary L-FABP level were not correlated with the changes in LDL-cholesterol level (R=0.19 and R= 0.15, respectively). The changes in UAE and L-FABP level were also not correlated with the changes in urinary 8-OHdG level (R=0.09, R=0.04, respectively). In the limited 23 patients with microalbuminuria, the changes in the values for UAE and L-FABP level were not correlated with the changes in LDL-cholesterol levels (R=0.09 and R= 0.13, respectively) as well as the changes in 8-OHdG level (R=0.02 and R= 0.10, respectively).

Among patient groups of the fluvastatin dose of 10 mg/day (n=6), 20 mg/day (n=25) and 30 mg/day (n=5), there were no differences in the changes for UAE [0.02±0.38, 0.19±0.19, 0.26±0.30 log(mg/g·creatinine) in the 10 mg/day, 20 mg/day and 30 mg/day groups, respectively] and urinary L-FABP level [0.04±0.56, 0.23±0.29, 0.73±0.37 log(μg/g·creatinine) in the 10 mg/day, 20 mg/day and 30 mg/day groups, respectively]. Between 27 patients receiving ACEIs or ARBs and the remaining 9 who did not receive them, there were no differences in the changes for UAE [0.16±0.28 vs 0.10±0.39 log(mg/g·creatinine)] and urinary L-FABP

Intern Med 50: 1273-1278, 2011 DOI: 10.2169/internalmedicine.50.4059

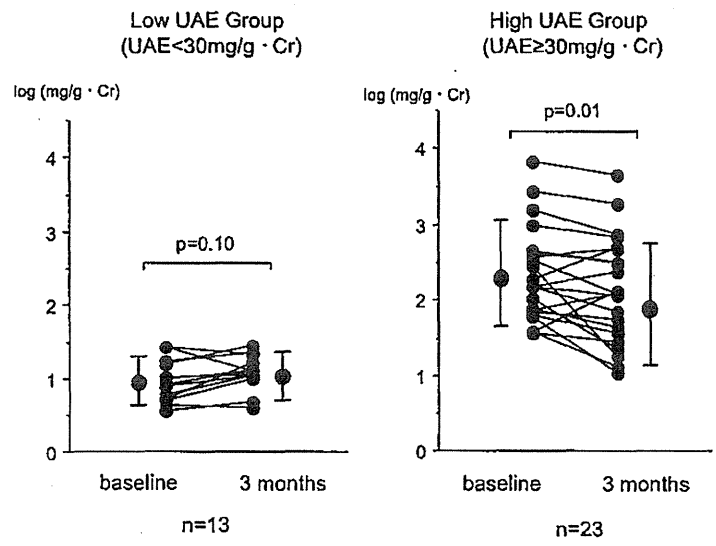

Figure 1. Change in urinary albumin excretion (UAE), separately shown in patients with (right panel) and without (left panel) microalbuminuria. The values were log-transformed.

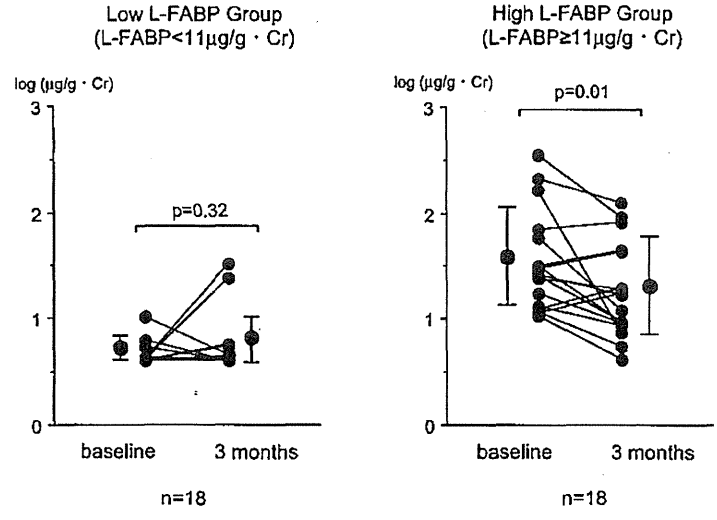

Figure 2. Changes in urinary liver-type fatty acid binding protein (L-FABP) separately shown in subgroups of high (right panel) and low (left panel) L-FABP level. The values were log-transformed.

level [0.55±0.66 vs 0.62±0.48 log(μg/g·creatinine)].

Discussion

In the FRET trial, in dyslipidemic patients with CKD we demonstrated that 3 months of treatment with fluvastatin reduced UAE in patients with microalbuminuria and also reduced urinary L-FABP level in the patients with microalbuminuria as well as in the subgroup of a high baseline L-FABP level. These results suggest that fluvastatin might be potentially effective to improve renal function in addition to its cholesterol lowering effect.

CKD is a potent risk factor for cardiovascular disease with an increased risk of cardiovascular events associated with even mild CKD. Cardiovascular death rather than progression to end-stage renal disease is a common outcome in patients with CKD (12). Abnormal lipid metabolism and dyslipidemia is considered to be an important promoter of renal dysfunction (13) with potential pathogenic mechanisms including not only theacceleration of atherosclerosis of the renal vasculature but also glomerular injury and tubulointerstitial damage. Although the underlying pathophysiological mechanisms are not yet fully understood, there are increasing numbers of data indicating that oxidative stress may mediate the lipid-induced renal damages. There is evidence that circulating lipids bind to and become trapped by cell membranes and extracellular matrix molecules (14), where they undergo oxidation increasing the formation of reactive oxygen species such as superoxide anion and hydrogen peroxide.

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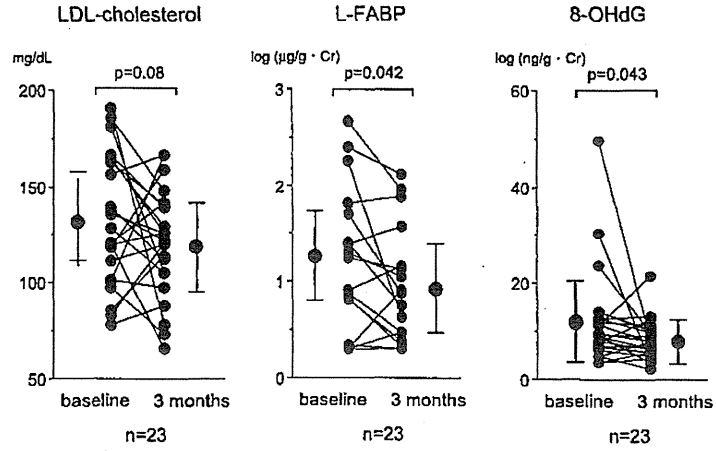

Figure 3. Changes in the levels of low density lipoprotein (LDL)-cholesterol, L-FABP and 8-hydroxydeoxyguanosine (8-OHdG) in the limited 23 patients. Although LDL-cholesterol level showed only the tendency for a decrease (left); the L-FABP (mid) and 8-OHdG (right) levels were significantly decreased. The values for L-FABP and 8-OHdG were log-transformed.

Recently, statins have been demonstrated to yield beneficial effects in different models of progressive renal failure. Although there is not yet a large interventional study on the effect of statin therapy in the progression of renal damage, there is evidence from post-hoc analyses to suggest that statins are likely to be effective in the treatment of renal disease (15). Statins are experimentally shown to have an antiproteinuric effect (16), as shown clinically in our FRET trial that demonstrated the improvement of microalbuminuria by fluvastatin. Since lipid lowering by statins reduces lipid trapping in renal tissues, the lipid lowering itself may contribute to renoprotective effects. However, some of the renoprotective effects of statins can be seen independent of the cholesterol reduction. In the FRET trial, the changes in the values for UAE and urinary L-FABP level were not correlated with the changes in LDL-cholesterol level not only in overall patients but also in the limited patients with microalbuminuria. These results suggest that fluvastatin might have a renoprotective effect beyond lipid lowering. Among various statins, fluvastatin is thought to be the most powerful anti-oxidant (5, 6). Different from other statins, fluvastatin has lipid-independent strong radical scavenging action and reduces superoxide anion formation both in vitro and in vivo (5). Fluvastatin has an indole ring in its structure, which is believed to be important for manifestation of these actions (17-19). L-FABP, which has high affinity for long-chain fatty acid oxidation products, may be an effective endogenous anti-oxidant. Since renal L-FABP reduces oxidative stress, ameliorating tubulointerstitial damage, urinary L-FABP, increased in association with renal dysfunction, is a potential marker of oxidative tubulointerstitial damage (20). In addition to the reduction of urinary L-FABP level not only in the high baseline L-FABP subgroup but also in the patients with microalbuminuria, our FRET trial showed the reduction of urinary 8-OHdG level, which is a marker for oxidative DNA damage, by 3 months of fluvastatin treatment in the patients with microalbuminuria. From our results, we can envision that the renoprotective effect of fluvastatin might be due to its anti-oxidative effect, although the changes in the urinary L-FABP level as well as UAE after fluvastatin treatment were not correlated with the changes in urinary 8-OHdG level not only in overall patients but also in the limited patients with microalbuminemia. Anyway, the results of FRET alone cannot determine the mechanism, by which fluvastatin ameliorates renal function.

Potential limitations

The FRET study has several potential limitations. This study was performed in a single arm no-controlled design with a small number of patients. Since comparisons with other statins were not performed, it was not elucidated whether the effects of fluvastatin on renoprotection are fluvastatin-specific effects or the class effects of statins. However, we believe it should be appreciated that this study was performed only by practitioners belonging to Saga Medical Association, because efforts of practitioners to prevent progression of CKD will be important for the improvement of cardiovascular mortality and morbidity of the CKD patients.

The authors state that they have no Conflict of Interest (COI).

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ORIGINAL ARTICLE

Antihypertensive treatment using an angiotensin receptor blocker and a thiazide diuretic improves patients' quality of life: The Saga Challenge Antihypertensive Study (S-CATS)

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The aim of the Saga Challenge Antihypertensive Study (S-CATS), a single-arm, prospective and multi-center trial, was to evaluate the effectiveness of combined antihypertensive treatment with losartan and hydrochlorothiazide (HCTZ). Enrolled in the study were a total of 161 patients with hypertension, who in spite of treatment with an angiotensin receptor blocker (ARB) alone or an ARB and calcium channel blocker (CCB), had not been able to reach blood pressure control goals set by the Japanese Society of Hypertension Guidelines (JSH 2004). The ARBs were replaced with a combination pill containing losartan (50 mg) and HCTZ (12.5 mg), and this treatment was continued for 3 months. This change in therapy resulted in significant decreases in systolic (158 ± 14 to 137 ± 15 mm Hg, $P<0.001$) and diastolic (85 ± 11 to 76 ± 10 mm Hg, $P<0.001$) blood pressure and heart rate (73 ± 3 to 72 ± 3) during the study. The patients' quality of life (QOL) score, the EuroQol 5 dimensions (EQ-5D) and the visual analog scale (VAS) (n=96; 70.0 (68.8–80.0) to 80.0 (70.0–90.0), $P<0.01$) all improved significantly. Another QOL score, the hypertension symptom score (HSS), which we originally developed for the S-CATS trial, decreased significantly (n=93; 4.0 (1.0–9.0) to 2.0 (1.0–8.0), $P<0.05$). The Pittsburgh sleep quality index (PSQI), which is a psychometric assessment of subjective sleep quality, also decreased significantly (n=45; 4.0 (2.0–7.0) to 3.0 (2.0–5.0), $P<0.05$). There was a significant correlation between a change in HSS (baseline value −3-months value) and a decrease in systolic blood pressure (n=93; R=0.241, $P<0.05$). These results suggest that an anti-hypertensive treatment combined with an ARB and a thiazide diuretic may improve patients' QOL, including sleep quality.
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Keywords: angiotensin receptor blocker; combination pill; quality of life; thiazide

INTRODUCTION

Hypertension is a prevalent and often asymptomatic chronic disease. The goal of antihypertensive treatment is to prevent associated complications and improve cardiovascular morbidity and mortality. To achieve these therapeutic goals, the most important issue is the blood pressure-lowering effect of a therapy. In addition, antihypertensive drugs, such as angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), have been shown to have protective pleiotropic effects in several organs, which may improve patient prognosis.[1,2] Despite effective medical therapy and evidence-based treatment guidelines for managing high blood pressure, uncontrolled hypertension remains common.[3,4] Low compliance with antihypertensive medication has been proposed as an important barrier to achieve hypertension control. To maintain treatment compliance or medication adherence, it is essential that patients experience an improvement in their quality of life (QOL) as a consequence of antihypertensive therapy.[5,6] However, there have been only a few studies that have specifically focused on patients' QOL during antihypertensive therapy.

The Saga Challenge Antihypertensive Study (S-CATS) is a single-arm, prospective and multi-center trial to evaluate the effectiveness of antihypertensive treatment with a combination pill containing losartan and hydrochlorothiazide (HCTZ). In this trial, we specifically focused on the effect of the losartan/HCTZ treatment on the patients' QOL.

METHODS

Study design

Local physicians and general practitioners at 12 hospitals and 30 clinics in Saga Prefecture, Japan participated in the S-CATS trial. Outpatients with hyperten-

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sion' were enrolled in this trial if, in spite of treatment with either an angiotensin receptor blocker (ARB) alone or combined therapy with an ARB and calcium channel blocker (CCB), their blood pressure control had not reached the goals set by the Japanese Society of Hypertension Guidelines (JSH 2004). Exclusion criteria included serious cardiac, cerebrovascular, hepatic or renal complications. Exclusion criteria did not include serum levels of K and creatinine. This study was approved by the ethical review board at Saga University Hospital.

In the treatment regimes of all the recruited patients, the ARBs were replaced with a losartan (50 mg)/HCTZ (12.5 mg) combination pill. This treatment was continued for an additional 3 months using the targets included in the 2004 JSH guidelines as the therapeutic goal.

Measurement of blood pressure

Measurements of blood pressure and heart rate were recorded in duplicate at each clinic visit and 24 ± 4 h after the previous administration of the study medication. The recordings were obtained after the patients had rested in a seated position for 5 min, at an interval of at least 1 min. At each visit, which occurred in the morning, office blood pressures were measured to the nearest 2 mm Hg in the same arm, using a mercury sphygmomanometer and an appropriately sized cuff. Home blood pressure measurement was recommended for participants using the upper-arm cuff device. Morning home blood pressure was measured twice with the subject with 3 min rest intervals: within 1 h after waking, after 1–2 min of rest following micturation, and before taking any antihypertensive drugs or eating breakfast. Just before going to bed, and again after 1–2 min of rest in a sitting position, evening home blood pressure was measured. The measurements of the home blood pressures were averaged over 7 days just before the hospital visit.

Assessment of quality of life

In the S-CATS trial, we specifically focused on the patients' QOL, using the EuroQol 5 dimensions (EQ-5D) score, the EuroQol visual analog scale (EQ-VAS), the hypertension symptom score (HSS) and the Pittsburgh sleep quality index (PSQI). The EQ-5D is a generic instrument for measuring health-related QOL, which has been developed and validated in a number of European countries.[7,8] The EQ-5D evaluates five dimensions of health status: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has three levels of assessment that include 'no problems', 'some problems' or 'severe problems'. This instrument yields 243 potential combinations of health states across the five dimensions. Dolan *et al.*[9] measured 42 of these

Symptoms

1 Headache or heaviness of head
2 Vertigo or tinnitus
3 Palpitation
4 Shortness of breath
5 Chest pain
6 Dizziness
7 Edema
8 Loss of concentration
9 Polyuria
10 Neck or shoulder stiffness

Frequency

None, 0; Occasionally, 1; Sometimes, 2; Often, 3; Always, 4

Figure 1 Calculation method of the hypertension symptom score (HSS). The HSS is a method for estimating patients' QOL, which we originally developed for the S-CATS trial. Each of the 10 listed hypertension-related symptoms was rated 0–4, for a five-grade scoring system. The HSS was calculated as the sum of each score for the 10 symptoms. Lower scores indicate better health, with a score of 40 being the worst and 0 being the best.

Table 1 Changes in blood chemistry findings during the 3-month observation period

	n	*Baseline*	*3 months*	*Value*
AST ($U\,l^{-1}$)	126	27 ± 13	27 ± 11	NS
ALT ($U\,l^{-1}$)	126	23 ± 14	23 ± 14	NS
BUN ($mg\,dl^{-1}$)	125	16.7 ± 4.1	18.7 ± 5.1	<0.001
Uric Acid ($mg\,dl^{-1}$)	121	5.2 ± 1.4	5.5 ± 1.4	<0.01
Creatinine ($mg\,dl^{-1}$)	124	0.77 ± 0.21	0.81 ± 0.24	<0.001
EGFR ($ml\,min^{-1}\,1.73\,m^{-2}$),	96	70.9 ± 19.3	66.7 ± 17.5	<0.001
⩾50	77	76.9 ± 12.8	71.7 ± 12.2	<0.001
<50	19	46.7 ± 8.6	46.4 ± 10.7	NS
Na ($mEq\,l^{-1}$)	121	141 ± 3	140 ± 5	NS
Cl ($mEq\,l^{-1}$)	120	104 ± 3	103 ± 3	<0.001
K ($mEq\,l^{-1}$)	120	4.2 ± 0.4	4.1 ± 0.4	<0.01
LDL-C ($mg\,dl^{-1}$)	96	116 ± 30	113 ± 32	NS
HDL-C ($mg\,dl^{-1}$)	118	57 ± 13	56 ± 15	NS
Triglyceride ($mg\,dl^{-1}$)	124	130 ± 79	128 ± 61	NS
Fasting Blood Glucose ($mg\,dl^{-1}$)	120	112 ± 45	111 ± 34	NS
HbA1c (%)	45	6.1 ± 1.0	6.2 ± 1.0	NS

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; Cl, chloride; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; K, potassium; LDL-C, low-density lipoprotein cholesterol; Na, sodium.

Table 2 Background of patients

Number of patients	183
Age	70 ± 12 y/o
Gender	
Male	45%
Female	55%
BMI	$24 \pm 5\,kg\,m^{-2}$
Abdominal circumference	85 ± 17 cm
Smoking	
Yes	12%
Previously	16%
Never	72%
Alcohol	
Daily	20%
Socially	21%
Never	59%
Complication	
Diabetes mellitus	25%
Hyperlipidemia	36%
Hyper uric acid	7%
Kidney disorder	16%
Pre-medication	
ARB only	45%
Combination of ARB and CCB	55%
Before switching over ARB (mean capacity)	
Losartan	19% (52 mg)
Candesartan	33% (8.5 mg)
Valsartan	22% (81 mg)
Telmisartan	15% (39 mg)
Olmesartan	11% (21 mg)

Abbreviation: BMI, body mass index.