References

1. Parving HH, Lehnert H, Bröchner-Mortensen J, Gomis R, Andersen S, Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study Group. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. N Engl J Med. 2001;345:870–8.
2. Viberti G, Wheeldon NM, MicroAlbuminuria Reduction With VALsartan (MARVAL) Study Investigators. Microalbuminuria reduction with valsartan in patients with type 2 diabetes mellitus: a blood pressure-independent effect. Circulation. 2002;106:672–8.
3. Lewis EJ, Hunsicker LG, Clarke WR, Collaborative Study Group, et al. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. N Engl J Med. 2001;345:851–60.
4. Brenner BM, Cooper ME, de Zeeuw D, RENAAL Study Investigators, et al. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med. 2001;345:861–9.
5. Haller H, Ito S, Izzo Jr JL, ROADMAP Trial Investigators, et al. Olmesartan for the delay or prevention of microalbuminuria in type 2 diabetes. N Engl J Med. 2011;364:907–17.
6. Smith DH, Dubiel R, Jones M. Use of 24-hour ambulatory blood pressure monitoring to assess antihypertensive efficacy: a comparison of olmesartan medoxomil, losartan potassium, valsartan, and irbesartan. Am J Cardiovasc Drugs. 2005;5:41–50.
7. Ogihara T, Kikuchi K, Matsuoka H, The Japanese Society of Hypertension Committee, et al. The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009). Hypertens Res. 2009;32:3–107.
8. Mancia G, De Backer G, Dominiczak A, The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC), et al. 2007 Guidelines for the Management of Arterial Hypertension. J Hypertens. 2007;25:1105–87.
9. Chobanian AV, Bakris GL, Black HR, Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, National Heart, Lung, and Blood Institute, National High Blood Pressure Education Program Coordinating Committee, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension. 2003;42:1206–52.
10. Jamerson K, Weber MA, Bakris GL, ACCOMPLISH Trial Investigators, et al. Benazepril plus amlodipine or hydrochlorothiazide for hypertension in high-risk patients. N Engl J Med. 2008;359:2417–28.
11. Bakris GL, Toto RD, McCullough PA, Rocha R, Purkayastha D, GUARD (Gauging Albuminuria Reduction With Lotrel in Diabetic Patients With Hypertension) Study Investigators. Effects of different ACE inhibitor combinations on albuminuria: results of the GUARD study. Kidney Int. 2008;73:1303–9.
12. Roggenenti P, Perna A, Loriga G, REIN-2 Study Group, et al. Blood-pressure control for renoprotection in patients with non-diabetic chronic renal disease (REIN-2): multicentre, randomised controlled trail. Lancet. 2005;365:939–46.
13. Bakris GL, Sarafidis PA, Weir MR, ACCOMPLISH Trial Investigators, et al. Renal outcomes with different fixed-dose combination therapies in patients with hypertension at high risk for cardiovascular events (ACCOMPLISH): a prespecified secondary analysis of a randomised controlled trial. Lancet. 2010;375:1173–81.
14. Aota Y, Morimoto S, Sakuma T, et al. Efficacy of an L- and N-type calcium channel blocker in hypertensive patients with neurovascular compression of the rostral ventrolateral medulla. Hypertens Res. 2009;32:700–5.
15. Kishi T, Hirooka Y, Konno S, Sunagawa K. Cilnidipine inhibits the sympathetic nerve activity and improves baroreflex sensitivity in patients with hypertension. Clin Exp Hypertens. 2009;31:241–9.
16. Fujita T, Ando K, Nishimura H, on behalf of the Cilnidipine versus Amlodipine Randomized Trial for Evaluation in Renal Disease (CARTER) Study Investigators, et al. Antiproteinuric effect of the calcium channel blocker cilnidipine added to renin-angiotensin inhibition in hypertensive patients with chronic renal disease. Kidney Int. 2007;72:1543–9.
17. Krespi PG, Makris TK, Hatzizacharias AN, et al. Moxonidine effect on microalbuminuria, thrombomodulin, and plasminogen activator inhibitor-1 levels in patients with essential hypertension. Cardiovasc Drugs Ther. 1998;12:463–7.

18. Mena-Martín FJ, Martín-Escudero JC, Simal-Blanco F, Carretero-Ares JL, Arzúa-Mouronte D, Castrodeza Sanz JJ, et al. Influence of sympathetic activity on blood pressure and vascular damage evaluated by means of urinary albumin excretion. J Clin Hypertens. 2006;8:619–24.
19. Shokoji T, Fujisawa Y, Kiyomoto H, et al. Effects of a new calcium channel blocker, azelnidipine, on systemic hemodynamics and renal sympathetic nerve activity in spontaneously hypertensive rats. Hypertens Res. 2005;28:1017–23.
20. Nada T, Nomura M, Koshiba K, Kawano T, Mikawa J, Ito S. Clinical study with azelnidipine in patients with essential hypertension. Antiarteriosclerotic and cardiac hypertrophy-inhibitory effects and influence on autonomic nervous activity. Arzneimittelforschung. 2007;57:698–704.
21. Eguchi K, Tomizawa H, Ishikawa J, et al. Effects of new calcium channel blocker, azelnidipine, and amlodipine on baroreflex sensitivity and ambulatory blood pressure. J Cardiovasc Pharmacol. 2007;49:394–400.
22. Ogihara T, Saruta T, Shimada K, Kuramoto K. A randomized, double-blind, four-arm parallel-group study of the efficacy and safety of azelnidipine and olmesartan medoxomil combination therapy compared with each monotherapy in Japanese patients with essential hypertension: the REZALT study. Hypertens Res. 2009;32:1148–54.
23. Shimada K, Ogihara T, Saruta T, REZALT Study Group. Effects of combination olmesartan medoxomil plus azelnidipine versus monotherapy with either agent on 24-hour ambulatory blood pressure and pulse rate in Japanese patients with essential hypertension: additional results from the REZALT study. Clin Ther. 2010;32:861–81.
24. Nakamura T, Sugaya T, Kawagoe Y, et al. Azelnidipine reduces urinary protein excretion and urinary liver-type fatty acid binding protein in patients with hypertensive chronic kidney disease. Am J Med Sci. 2007;333:321–6.
25. Ogawa S, Mori T, Nako K, Ito S. Combination therapy with renin-angiotensin system inhibitors and the calcium channel blocker azelnidipine decreases plasma inflammatory markers and urinary oxidative stress markers in patients with diabetic nephropathy. Hypertens Res. 2008;31:1147–55.
26. Matsuo S, Imai E, Horio M, Collaborators developing the Japanese equation for estimated GFR, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis. 2009;53:982–92.
27. Katayama S, Kawamori R, Iwamoto Y, Saito I, Kuramoto K, on behalf of the ATTEST Study Group. In half of hypertensive diabetics, co-administration of a calcium channel blocker and an angiotensin-converting enzyme inhibitor achieved a target blood pressure of <130/80 mmHg: the Azelnidipine and Temocapril in hypertensive patients with type 2 diabetes (ATTEST) study. Hypertens Res. 2008;31:1499–508.
28. Magee GM, Bilous RW, Cardwell CR, Hunter SJ, Kee F, Fogarty DG. Is hyperfiltration associated with the future risk of developing diabetic nephropathy? A meta-analysis. Diabetologia. 2009;52:691–7.
29. Epstein M. Calcium antagonists and renal disease. Kidney Int. 1998;54:1771–84.
30. Uchino K, Nishikimi T, Frohlich ED. Alpha 1-adrenergic receptor blockade reduces afferent and efferent glomerular arteriolar resistances in SHR. Am J Physiol Regul Integr Comp Physiol. 1991;261:R576–80.
31. Fujimoto S, Satoh M, Nagasu H, Horike H, Sasaki T, Kashihara N. Azelnidipine exerts renoprotective effects by improvement of renal microcirculation in angiotensin II infusion rats. Nephrol Dial Transplant. 2009;24:3651–8.
32. Inaba S, Iwai M, Tomono Y, et al. Prevention of vascular injury by combination of an AT1 receptor blocker, olmesartan, with various calcium antagonists. Am J Hypertens. 2009;22:145–50.
33. Matsui Y, Eguchi K, O'Rourke MF, et al. Differential effects between a calcium channel blocker and a diuretic when used in combination with angiotensin II receptor blocker on central aortic pressure in hypertensive patients. Hypertension. 2009;54:716–23.

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-

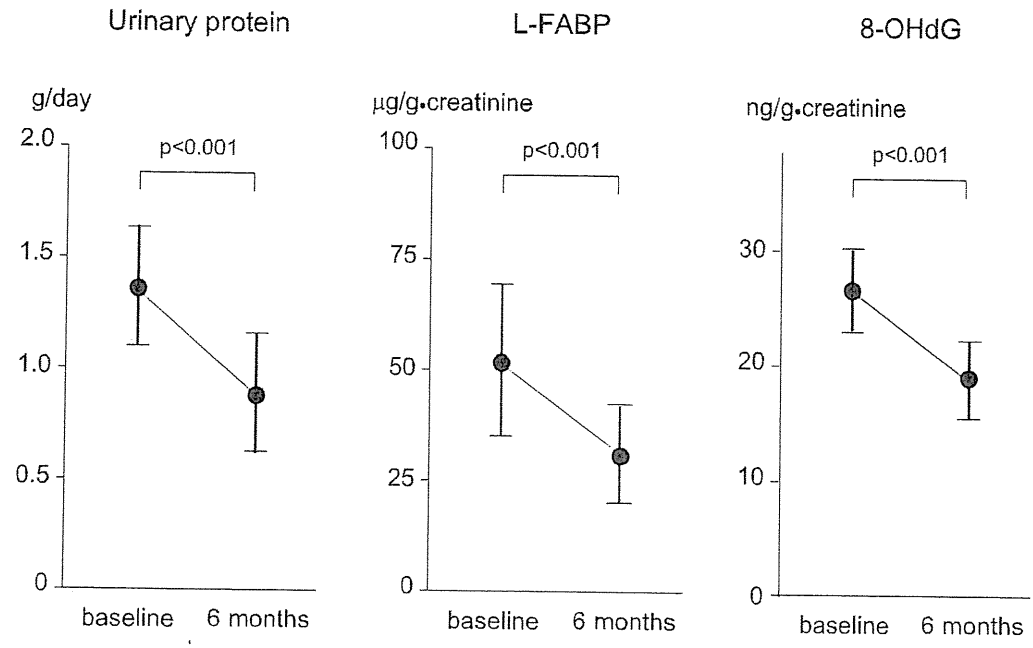

Figure 1. Changes in urinary protein excretion, urinary L-FABP level and urinary 8-OHdG levels before and after 6 months of EPO treatment.

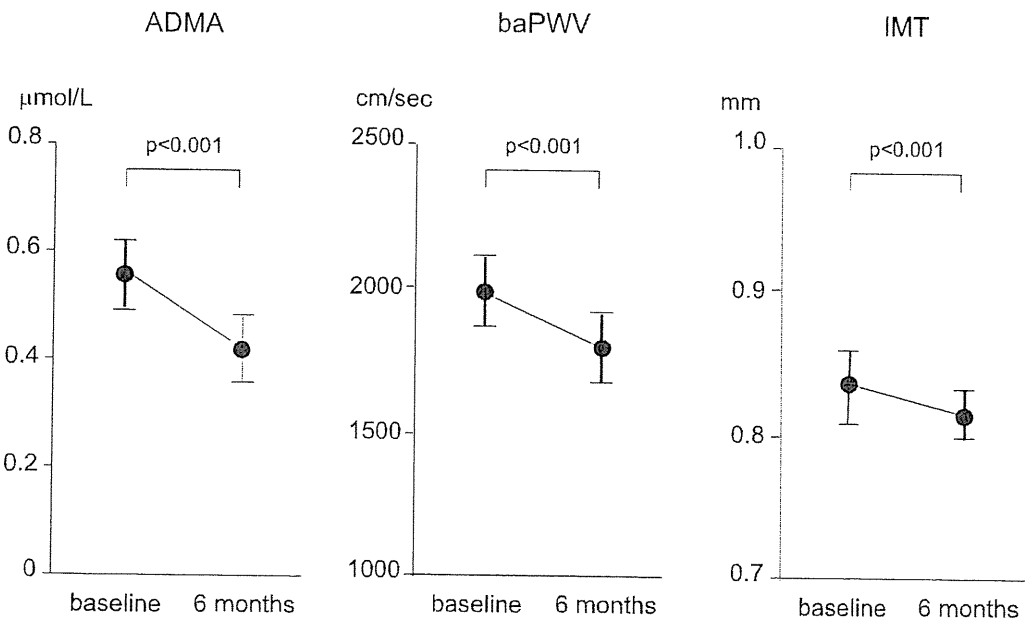

Figure 2. Changes in serum ADMA level, baPWV and IMT before and after 6 months of EPO treatment.

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

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-

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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References

1. Toyeux-Faure M. Cellular protection by erythropoietin: New therapeutic implications? J Pharmacol Exp Therapy **323**: 759-762, 2007.
2. Gluhovschi G, Gluhovschi C, Bob F, et al. Multiorgan-protective actions of blockers of the renin-angiotensin system, statins and erythropoietin: common pleiotropic effects in reno-, cardio- and neuroprotection. Acta Clin Belg **63**: 152-169, 2008.
3. Nakamura T, Sato E, Fujiwara N, et al. Co-administration of ezetimibe enhances proteinuria-lowering effects of pitavastatin in chronic kidney disease patients partly via a cholesterol-independent manner. Pharmacol Res **61**: 58-61, 2010.
4. Tanaka T, Doi K, Maeda-Mamiya R, et al. Urinary L-type fatty acid-binding protein can reflect renal tubulointerstitial injury. Am J Pathol **174**: 1203-1211, 2009.
5. Nakamura T, Sugaya T, Kawagoe Y, et al. Azelnidipine reduces urinary protein excretion and urinary liver-type fatty acid binding protein in patients with hypertensive chronic kidney disease. Am J Med Sci **333**: 321-326, 2007.
6. Nakamura T, Sato E, Fujiwara N, et al. Ezetimibe decreases serum levels of asymmetric dimethylarginine (ADMA) and ameliorates renal injury in non-diabetic chronic kidney disease patients in a cholesterol-independent manner. Pharmacol Res **60**: 525-528, 2009.
7. Kasap B, Soylu A, Kuralay F, et al. Protective effect of EPO on oxidative renal injury in rats with cyclosporine nephrotoxicity. Pediatr Nephrol **23**: 1991-1999, 2008.
8. Bi B, Guo J, Marlier A, Lin SR, Cantley LG. Erythropoietin expands a stromal cell population that can mediate renoprotection. Am J Physiol Renal Physiol **295**: F1017-F1021, 2008.
9. Wang MC, Tsai WC, Chen JY, Huang JJ. Stepwise increase in arterial stiffness corresponding with the stages of chronic kidney disease. Am J Kidney Dis **45**: 494-501, 2005.
10. Preston E, Ellis MR, Kulinskaya E, Davies AH, Brown A. Association between carotid artery intima-media thickness and cardiovascular risk factors in CKD. Am J Kidney Dis **46**: 856-862, 2005.
11. Ueda S, Yamagishi S, Matsumoto Y, et al. Involvement of asymmetric dimethylarginine (ADMA) in glomerular capillary loss and sclerosis in a rat model of chronic kidney disease (CKD). Life Sci **84**: 853-856, 2009.
12. Kielstein JT, Fliser D. The past, presence and future of ADMA in nephrology. Nephrol Ther **3**: 47-54, 2007.
13. Tagore R, Ling LH, Yang H, Daw HY, Chan YH, Sethi SK. Natriuretic peptides in chronic kidney disease. Clin J Am Soc Nephrol **3**: 1644-1651, 2008.
14. Belov VV, Il'icheva OE. The role of brain natriuretic peptide in diagnosis of diastolic myocardial dysfunction in patients with predialysis chronic kidney disease. Kardiologiia **47**: 10-13, 2007.
15. Goto S, Yoshida K, Kita T, Fujii H, Fukagawa M. Uremic toxins and oral adsorbents. Ther Apher Dial **15**: 132-134, 2011.
16. Ayus JC, Go AS, Valderrabapo F, et al. Effects of erythropoietin

on left ventricular hypertrophy in adults with severe chronic renal failure and hemoglobin <10 g/dL. Kidney Int **68**: 788-795, 2005.
17. Imai E, Horio M, Nitta K, et al. Modification of the modification of Diet in Renal Disease (MDRD) study equation for Japan. Am J Kidney Dis **50**: 927-937, 2007.
18. Kamijo A, Kimura K, Sugaya T, et al. Urinary fatty acid-binding protein as a new clinical marker of the progression of chronic renal disease. J Lab Clin Med **143**: 23-30, 2004.
19. Saito S, Yamauchi H, Hasui Y, Kurashige J, Ochi H, Yoshida K. Quantitative determination of urinary 8-hydroxyguanosine (8-OHdG) by using ELISA. Res Commun Mol Pathol Pharmacol **107**: 39-44, 2000.
20. Ueda S, Kato S, Matsuoka H, et al. Regulation of cytokine-induced nitric oxide synthesis by asymmetric dimethylarginine. Cir Res **92**: 226-233, 2003.
21. Matsuguma K, Ueda S, Yamagishi S. et al. Molecular mechanism for elevation of asymmetric dimethylarginine and its role for hypertension in chronic kidney disease. J Am Soc Nephrol **17**: 2176-2183, 2006.
22. Nakamura T, Inoue T, Suzuki T, Ueda Y, Koide H, Node K. Comparison of renal and vascular protective effects between telmisartan and amlodipine in hypertensive patients with chronic kidney disease with mild renal insufficiency. Hypertens Res **31**: 841-850, 2008.
23. Nakamura T, Suzuki T, Kawagoe Y, Koide H. Polymyxin B-immobilized fiber hemoperfusion attenuates increased plasma atrial natriuretic peptide and brain natriuretic levels in patients with septic shock. ASAIO J **54**: 210-213, 2008.
24. Shibazaki K, Kimura K, Okada Y, et al. Plasma brain natriuretic peptide as an independent predictor of in-hospital mortality after acute ischemic stroke. Intern Med **48**: 1601-1606, 2009.
25. Bahlmann FH, Fliser D. Erythropoietin and renoprotection. Curr Opin Nephrol Hypertens **18**: 15-20, 2009.
26. Srisawat N, Manotham K, Eiam-Ong S, Katavetin P, Praditpornsilpa K, Eiam-Ong S. Erythropoietin and its non-erythropoietic derivative: Do they ameliorate renal tubulointerstitial injury in ureteral obstruction?. Int J Urol **15**: 1011-1017, 2008.
27. Breggia AC, Wojchowski DM, Himmelfarb J. JAK2/Y343/STAT5 signaling axis is required for erythropoietin-mediated protection against ischemic injury in primary renal tubular epithelial cells. Am J Physiol Renal Physiol **295**: F1689-F1695, 2008.
28. Katavetin P, Tungsanga K, Eiam-Ong S, Nangaku M. Antioxidative effects of erythropoietic. Kidney Int Suppl **107**: S10-S15, 2007.
29. Ueda S, Yamagishi S, Matsumoto Y, Fukami K, Okuda S. Asymmetric dimethylarginine (ADMA) is a novel emerging risk factor for cardiovascular disease and the development of renal injury in chronic kidney disease. Clin Exp Nephrol **11**: 115-121, 2007.
30. Sharma M, Zhou Z, Miura H. et al. ADMA injures the glomerular filtration barrier: role of nitric oxide and superoxide. Am J Physiol Renal Physiol **296**: F1386-F1395, 2009.
31. Furuki K, Adachi H, Matsuoka H. Plasma levels of asymmetric dimethylarginine (ADMA) are related to intima-media thickness of the carotid artery: an epidemiological study. Atherosclerosis **191**: 206-210, 2007.
32. Zhang L, Zhao F, Yang Y, et al. Association between carotid artery intima-media thickness and early-stage CKD in a Chinese population. Am J Kidney Dis **49**: 786-792, 2007.
33. Pawiak K, Pawiak D, Mysliwiec M. Long-term erythropoietin therapy decreases CC-chemokine levels and intima-media thickness in hemodialyzed patients. Am J Nephrol **26**: 497-502, 2006.
34. Hirashiki A, Murohara T. The impact of pulse wave velocity in a Japanese population with metabolic syndrome. Hypertens Res **32**: 1045-1046, 2009.
35. Dhar S, Pressman GS, Subramanian S, et al. Natriuretic peptides and heart failure in the patient with chronic kidney disease: A review of current evidence. Postgrad Med J **85**: 299-302, 2009.

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-

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

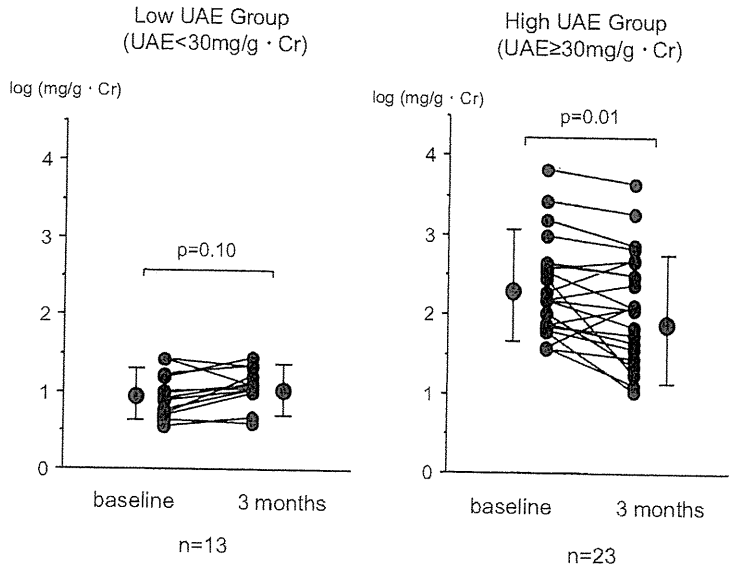

Figure 1. Change in urinary albumin excretion (UAE), separately shown in patients with (right panel) and without (left panel) microalbuminuria. The values were log-transformed.

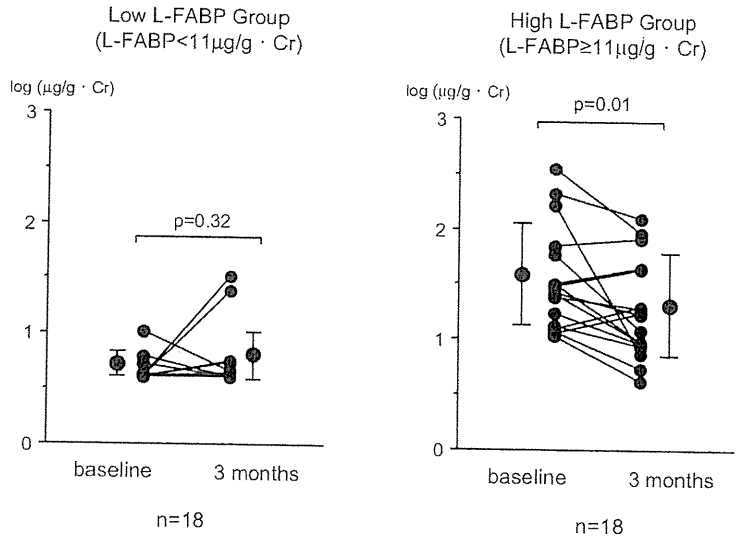

Figure 2. Changes in urinary liver-type fatty acid binding protein (L-FABP) separately shown in subgroups of high (right panel) and low (left panel) L-FABP level. The values were log-transformed.

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.

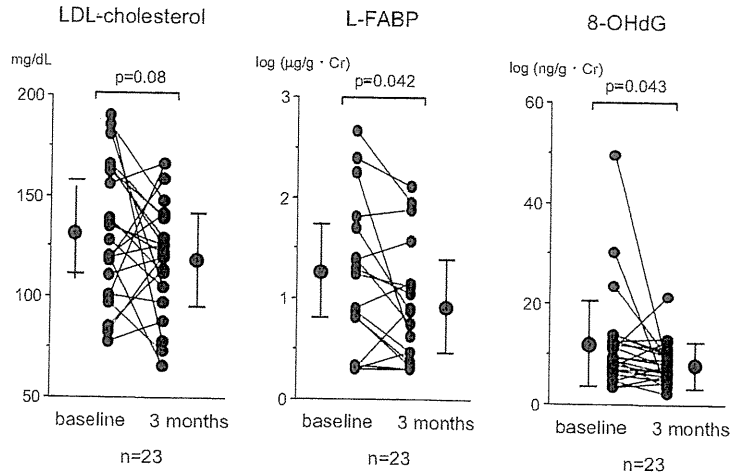

Figure 3. Changes in the levels of low density lipoprotein (LDL)-cholesterol, L-FABP and 8-hydroxydeoxyguanosine (8-OHdG) in the limited 23 patients. Although LDL-cholesterol level showed only the tendency for a decrease (left); the L-FABP (mid) and 8-OHdG (right) levels were significantly decreased.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

References

1. Parikh NI, Hwang SJ, Larson MG, Meigs JB, Levy D, Fox CS. Cardiovascular disease risk factors in chronic kidney disease: overall burden and rates of treatment and control. Arch Intern Med **166**: 1884-1891, 2006.
2. Schaeffner ES, Kurth T, Curhan GC, et al. Cholesterol and the risk of renal dysfunction in apparently healthy men. J Am Soc Nephrol **14**: 2084-2091, 2003.
3. Chan CM. Hyperlipidemia in chronic kidney disease. Ann Acad Med Singapore **34**: 31-35, 2005.

4. Sandhu S, Wiebe N, Fried LF, Tonelli M. Statins for improving renal outcomes: a meta-analysis. J Am Soc Nephrol **17**: 2006-2016, 2006.
5. Suzumura K, Yasuhara M, Narita H. Superoxide anion scavenging properties of fluvastatin and its metabolites. Chem Pharm Bull **47**: 1477-1480, 1994.
6. Inoue T, Hayashi M, Takayanagi K, Morooka S. Lipid lowering therapy with fluvastatin inhibits oxidative modification of low density lipoprotein and improves vascular endothelial function in hypercholesterolemic patients. Atherosclerosis **160**: 369-376, 2002.
7. Malle E, Woenckhous C, Waeg G, Esterbauer H, Gröne EF, Gröne HJ. Immunological evidence for hypochlorite-modified proteins in human kidney. Am J Pathol **150**: 603-615, 1997.
8. Kamijo A, Kimura K, Sugaya T, et al. Urinary fatty acid-binding protein as a new clinical marker of the progression of chronic renal disease. J Lab Clin Med **143**: 23-30, 2004.
9. Kamijo A, Sugaya T, Hikawa A, et al. Clinical evaluation of urinary excretion of liver-type fatty acid binding as a marker for the monitoring of chronic kidney disease: A multicenter trial. J Lab Clin Med **145**: 125-133, 2005.
10. Nakamura T, Sugaya T, Ebihara I, Koide H. Urinary liver-type fatty acid-binding protein: discrimination between IgA nephropathy and thin basement membrane nephropathy. Am J Nephrol **25**: 447-450, 2005.
11. Saito S, Yamauchi H, Hasui Y, Kurashige J, Ochi H, Yoshida K. Quantitive determination of urinary 8-hydroxy deoxyguanosine (8-OHdG) by using ELISA. Res Commun Mol Pathol Pharmacol **107**: 39-44, 2000.
12. Levey AS, Eckardt KU, Tsukamoto Y, et al. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcome (KDIGO). Kidney Int **67**: 2089-2100, 2005.
13. Schaeffner ES, Kurth T, Curhan GC, et al. Cholesterol and the risk of renal dysfunction in apparently healthy men. J Am Soc Nephrol **14**: 2084-2091, 2003.
14. Abrass CK. Cellular lipid metabolism and the role of lipids in progressive renal disease. Am J Nephrol **24**: 46-53, 2004.
15. Trevisan R, Dodesini AR, Lepore G. Lipids and renal disease. J Am Soc Nephrol **17**: S145-S147, 2006.
16. Zoja C, Corna D, Rottoli D, et al. Effect of combining ACE inhibitor and statin in severe experimental nephropathy. Kidney Int **61**: 1635-1645, 2002.
17. Suzumura K, Yasuhara M, Tanaka K, Odawara A, Narita H, Suzuki T. An in vitro study of the hydroxyl radical scavenging property of fluvastatin, an HMG-CoA redactase inhibitor. Chem Pharm Bull **47**: 1010-1012, 1999.
18. Yamamoto A, Hoshi K, Ichihara K. Fluvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase, scavenges free radicals and inhibits lipid peroxidation in rat liver microsome. Eur J Pharmacol **361**: 143-149, 1998.
19. Rikitake Y, Kawashima S, Takeshita S, et al. Anti-oxidative properties of fluvastatin, an HMG-CoA reductase inhibitor, contribute to prevention of atherosclerosis in cholesterol-fed rabbits. Atherosclerosis **154**: 87-96, 2001.
20. Kamijo-Ikemori A, Sugaya T, Obama A, et al. Liver-type fatty acid-binding protein attenuates renal injury induced by unilateral ureteral obstruction. Am J Pathol **169**: 1107-1117, 2006.

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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 ± 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.

health states in a representative sample of the United Kingdom's general population, using the time trade-off (TTO) method.[10] Based on these evaluations, utility scores can be deduced using an additive function. Utility scores may vary between −0.59 (worst health) and 1.00 (perfect health). In addition to the five dimensions, the EuroQol consists of an EQ-VAS ranging from 0 (worst imaginable health state) to 100 (best imaginable health state).[11] The HSS is a method of estimating patients' QOL, which we originally developed for the S-CATS trial. This score is calculated as the sum of scores (grades 1–5) of 10 hypertension-related symptoms (Figure 1).

The PSQI is a self-administered questionnaire to assess subjective sleep quality during the previous month.[12] The self-rated items of the PSQI generate seven component scores (range of subscale scores, 0–3) on sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleeping medications and daytime dysfunction. The sum of these seven component scores yields one global score of subjective sleep quality (range of scores, 0–21), with higher scores representing poorer subjective sleep quality. The psychometric properties of the PSQI have been confirmed in previous studies.[12,13]

Data analysis

The data were collected before (baseline) and 3 months after the replacement of ARBs with the losartan/HCTZ combination pill therapy. The values were expressed as mean ± s.d. for parametric data and as median value and interquartile range for non-parametric data. The statistical analyses were performed using the paired *t*-test for parametric data and the Wilcoxon rank-sum test for non-parametric data. The correlation between the two variables was examined using the Spearman rank correlation coefficient. A *P*-value <0.05 was considered to be statistically significant.

RESULTS

We analyzed the data from 161 patients (72 men and 89 women, aged 70 ± 11 yrs) who were followed during the 3-month observation period. The characteristics of these patients are summarized in Table 1. There were no major adverse effects in this study. The baseline antihypertensive medications that the patients had been taking before

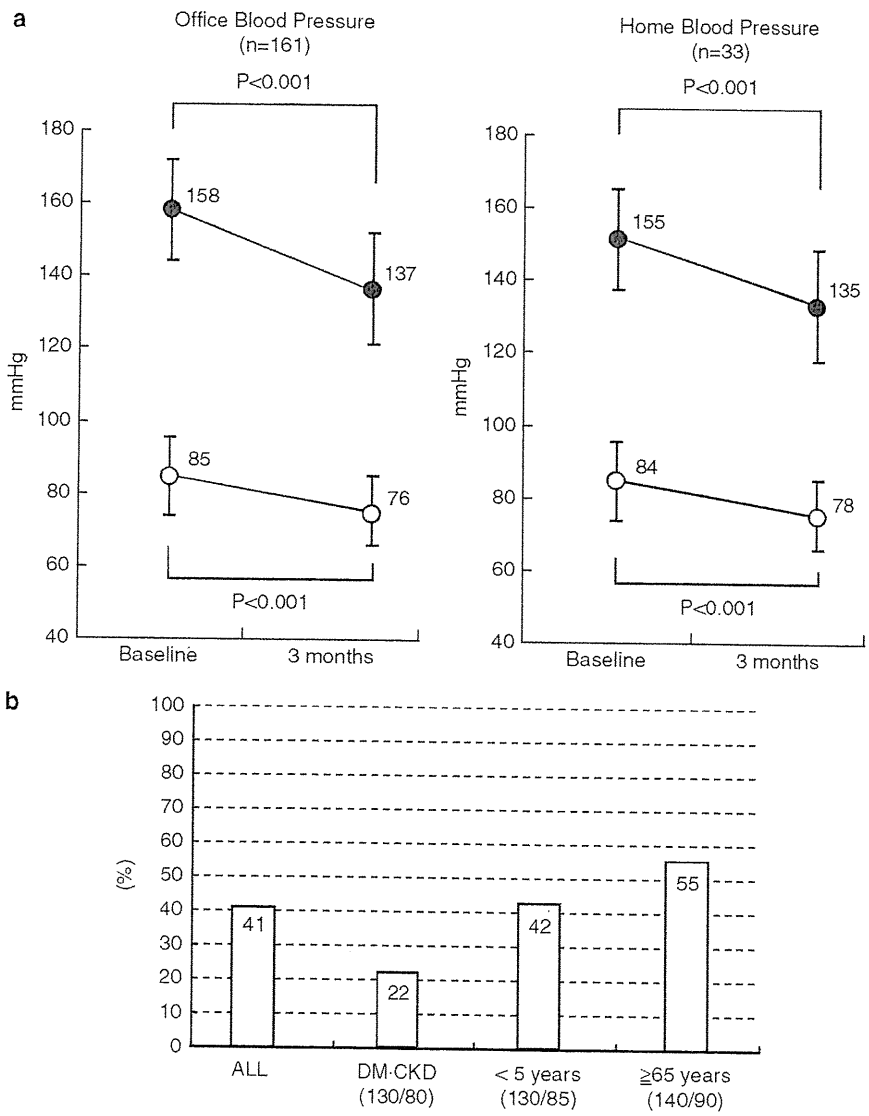

Figure 2 (a) Changes in blood pressure during the 3-month observation period after the replacement of ARBs with the losartan (50 mg)/HCTZ (12.5 mg) combination pill. After 3 months of treatment, the office blood pressures of all 161 patients decreased significantly (left). Home blood pressures also decreased significantly in 33 patients for whom the data were obtained both at baseline and 3 months after treatment with the combination pill (right). • indicates systolic blood pressure, O indicates diastolic blood pressure (**b**) The guideline achievement rate shows the responders' blood pressure-lowering characteristics compared with those of the non-responders after changing the medication. CKD, chronic kidney disease; DM, diabetes mellitus.

the medication exchange were ARBs alone in 72 patients (45%) and ARBs with CCBs in 89 patients (55%). The baseline ARBs were losartan in 31 patients (19%), candesartan in 53 (33%), valsartan in 35 (22%), telmisartan in 24 (15%) and olmesartan in 18 (11%) (Table 2). During the 3-month observation period, office blood pressure decreased significantly (systolic 158 ± 14 to 137 ± 15 mm Hg, $P<0.001$; diastolic 85 ± 11 to 76 ± 10 mm Hg, $P<0.001$) (Figure 2a, left), and heart rate did not change significantly (73 ± 3 to 72 ± 3). Home blood pressure also decreased significantly (systolic 155 ± 21 to 135 ± 15 mm Hg, $P<0.001$; diastolic 84 ± 13 to 78 ± 11 mm Hg, $P<0.001$) (Figure 2a, right). As the result of adhering to the guidelines of the Japanese Society of Hypertension, there were difficulties in using the drug combination to treat the patients with complications of diabetic mellitus and chronic kidney disease (Figure 2b). The blood pressure-lowering effect was similar between the patients taking ARBs alone and those taking ARBs with CCBs as the baseline medications (Figure 3). In addition, the blood pressure-lowering effect was independent of the differences in baseline ARBs (Figure 4).

Changes in blood biochemistry profiles are shown in Table 1. Significant increases in serum levels of blood urea nitrogen (16.7 ± 4.1 to 18.7 ± 5.1 mg dl^{-1}, $P<0.001$), uric acid (5.2 ± 1.4 to 5.5 ± 1.4 mg dl^{-1}, $P<0.01$) and creatinine (0.77 ± 0.22 to 0.81 ± 0.24 mg dl^{-1}, $P<0.001$) were evident during the observation period. Estimated glomerular filtration rate (eGFR) was calculated in 96 patients according to the Japanese Society of Nephrology Chronic Kidney Disease Practice Guide, and it was shown to decrease significantly from 70.9 ± 19.3 to 66.7 ± 17.5 ml min^{-1} 1.73 m^{-2} ($P<0.001$). Of these 96 patients, 77 patients with an eGFR ≥ 50 ml min^{-1} 1.73 m^{-2} showed a significant decrease (76.9 ± 12.8 to 71.7 ± 12.2, $P<0.001$). In the remaining 19 patients with an eGFR < 50 ml min^{-1} 1.73 m^{-2}, the eGFR value did not change significantly (46.7 ± 8.6 to 46.4 ± 10.7). Serum chloride and potassium levels decreased significantly (104 ± 3 to 103 ± 3 mEq l^{-1}, $P<0.001$, 4.2 ± 0.4 to 4.1 ± 0.4 mEq l^{-1}, $P<0.01$, respectively).

The EQ-5D index values analyzed in 95 patients increased significantly during the study from 1.00 (0.71–1.00) to 1.00 (0.76–1.00) ($P<0.01$). Of these 95 patients, 20 patients with an EQ-5D < 0.7 showed a significant increase from 0.65 (0.59–0.69) to 0.67 (0.62–0.76) ($P<0.05$). The score did not change significantly in the remaining 75 patients with an EQ-5D ≥ 0.7 (1.00 (0.77–1.00) to 1.00 (1.00–1.00)). The VAS analyzed in 96 patients increased significantly from 70.0 (68.8–80.0) to 80.0 (70.0–90.0) ($P<0.01$). Of these 96 patients, 50 patients with a VAS < 70 showed a significant increase from 70.0 (56.3–70.0) to 70.0 (60.0–80.0), ($P<0.001$). However, in the remaining 46 patients with a VAS ≥ 70, the score did not change significantly (80.0 (80.0–90.0) to 85.0 (80.0–90.0)). The PSQI analyzed in 45 patients decreased significantly from 4.0 (2.0–7.0) to 3.0 (2.0–5.0) ($P<0.05$). Of these 45 patients, 13 patients with a PSQI ≥ 5.5 showed a significant decrease (8.0 (7.0–9.0) to 7.0 (5.0–7.0), $P<0.05$), whereas the score did not change significantly in the remaining 32 patients with a PSQI < 5.5 (3.0 (1.0–4.0) to 2.5 (1.0–4.0)) (Table 3). Finally, the HSS analyzed in 93 patients decreased significantly (4.0 (1.0–9.0) to 2.0 (1.0–8.0), $P<0.05$) (Figure 5a). In these 93 patients, the change in HSS (the baseline value minus the 3-month value) correlated with systolic blood pressure ($R=0.241$, $P=0.0195$) (Figure 5b).

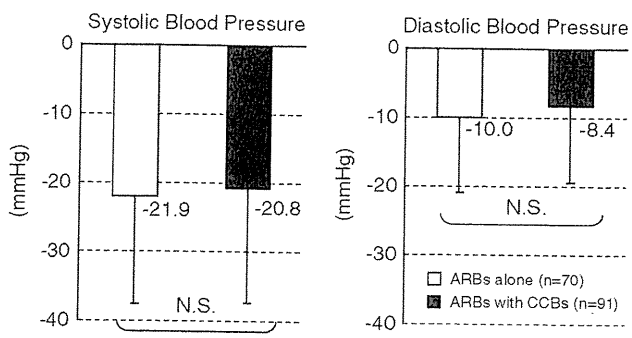

Figure 3 Comparison of the blood pressure-lowering effects between the patients taking ARBs alone and those taking ARBs with CCBs as the baseline medications. The effects were similar between two groups.

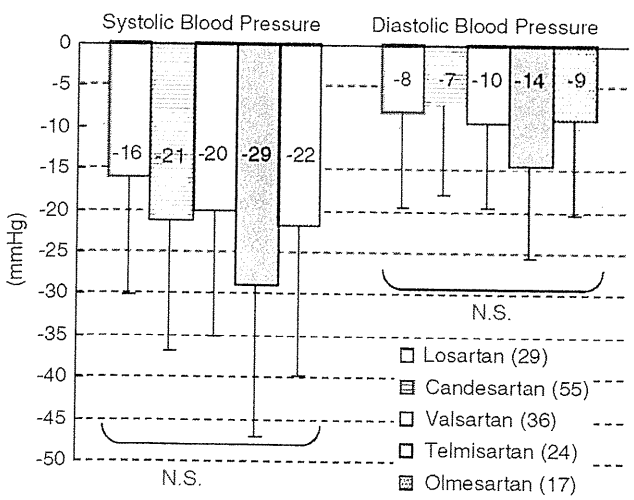

Figure 4 Comparison of blood pressure-lowering effects among the patient groups for each of baseline ARBs. The effects were similar among the groups.

DISCUSSION

In this study of hypertensive patients, the replacement of an ARB with a combination pill containing losartan and HCTZ achieved a mean reduction in systolic blood pressure of 21 mm Hg and in diastolic blood pressure of 9 mm Hg. In addition, all the QOL scores we

Table 3 Changes in quality of life (QOL) during the 3-month observation period

	n	*Baseline*	*3 months*	*Value*
EuroQol 5 dimensions (EQ 5D)				
	95	1.00 (0.71–1.00)	1.00 (0.76–1.00)	$P<0.01$
<7	20	0.65 (0.59–0.69)	0.67 (0.62–0.76)	$P<0.05$
≥7	75	1.00 (0.77–1.00)	1.00 (1.00–1.00)	NS
Visual analog scale (VAS)				
	96	70.0 (68.8–80.0)	80.0 (70.0–90.0)	$P<0.01$
<70	50	70.0 (56.3–70.0)	70.0 (60.0–80.0)	$P<0.001$
≥70	46	80.0 (80.0–90.0)	85.0 (80.0–90.0)	NS
Pittsburgh sleep quality index (PSQI)				
	45	4.0 (2.0–7.0)	3.0 (2.0–5.0)	$P<0.05$
<5.5	13	3.0 (1.0–4.0)	2.5 (1.0–4.0)	NS
≥5.5	32	8.0 (7.0–9.0)	7.0 (5.0–7.0)	$P<0.05$

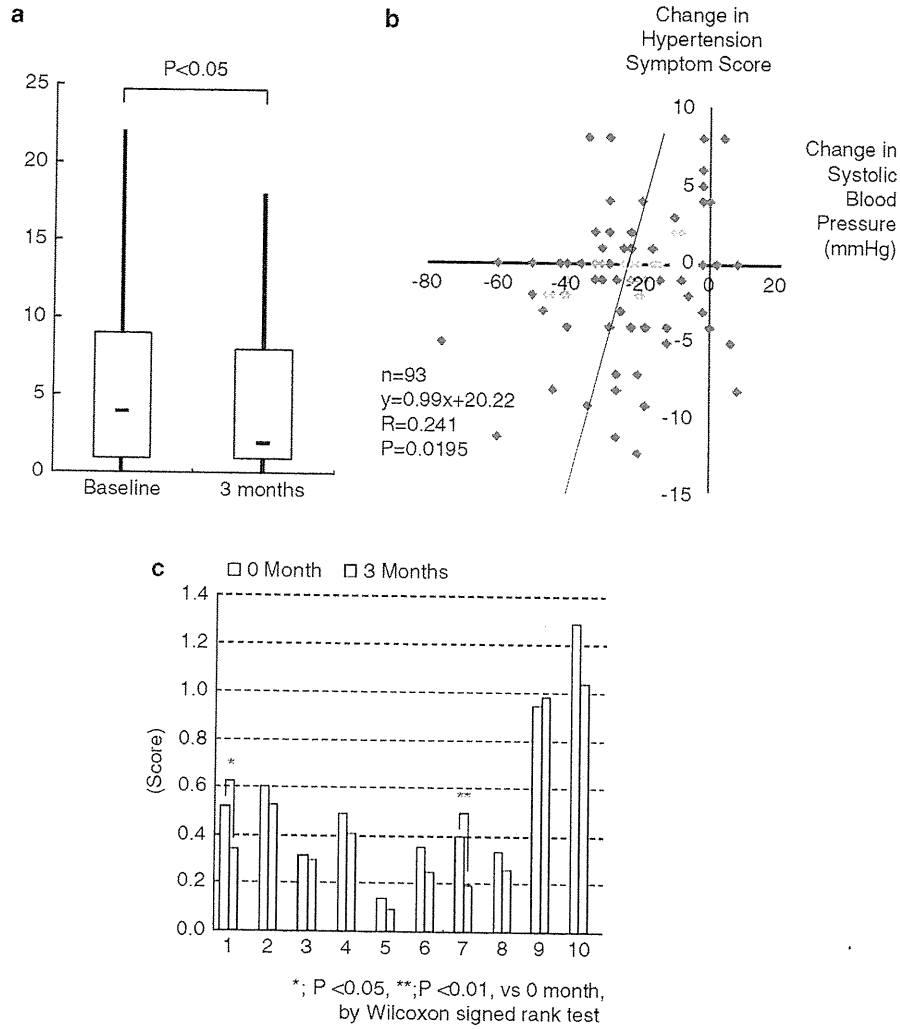

Figure 5 (**a**) Change in HSS after 3 months of treatment with the losartan (50 mg)/HCTZ (12.5 mg) combination pill (n=93). Overall, HSS decreased significantly (Wilcoxon rank-sum test). The solid line represents the median value; the box, the interquartile range; and the error bar, the 95% confidence interval. (**b**) Graph showing the positive correlation between the change in HSS and systolic blood pressure during the 3-month observation period following the replacement of ARBs with the losartan (50 mg)/HCTZ (12.5 mg) combination pill. (**c**) Self-assessment survey completed by patients with hypertension shows detailed examples of the effects of an improved hypertension symptom score. 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 and 10: neck or shoulder stiffness.

measured in the study (that is EQ-5D, VAS, HSS and PSQI) improved after treatment with the losartan/HCTZ combination pill.

Patients seen in primary care settings often present with unspecific symptoms irrespective of the underlying medical problem. Many of these symptoms are non-specific. Patients with hypertension frequently report symptoms that are also reported by normotensive patients.[14] Hypertension is usually described as asymptomatic in the absence of significant target organ damage and concomitant disease, although cognitive changes, mood alterations and general symptoms, such as dizziness and headache, have been described.[15–19] Some symptoms are unique to the effects of antihypertensive drugs, whereas others overlap with symptoms described as or attributed to the hypertensive disease itself or are inseparable from those observed throughout the primary care population.[14]

The ultimate goal of antihypertensive treatment is the reduction of cardiovascular mortality and morbidity. To achieve this goal, treatment compliance and medication adherence need to be maintained. If patients taking antihypertensive drugs do not feel a reduction in symptoms, it is unlikely that they will comply or adhere to the treatment. Symptoms, whether disease- or treatment-induced, may impair the health-related QOL of patients. QOL refers to the physical, emotional and social impact of a disease and its treatments[20,21] and is distinct from the physiological measures of disease.[20–23] Estimation of QOL may assess the impact of a disease and its treatment from a patient's perspective to a greater extent than it assesses conventional clinical symptoms. To estimate the patients' QOL, several scoring systems in the form of questionnaires on various health-related factors have been developed and validated. The EQ-5D is commonly used to measure health-related QOL and has been shown to be responsive, internally consistent and reliable in the normal population and other patient groups.[9,10] The VAS is also used to measure health-related QOL. Although it is not as sensitive as other measures, the VAS does

have considerable merit because of its ease of application, which makes the collection of panel data feasible.[11] To date, there have been no reports that have specifically evaluated EQ-5D or VAS in the treatment of hypertension. However, another method for evaluating QOL, the 36-item Short Form questionnaire (SF-36), has been used in hypertensive patient populations. Because all these QOL scores represent health-related evaluations, we used our original QOL score from the HSS questionnaire in the present study. This questionnaire focuses on comparatively specific hypertension-related symptoms, calculated as the sum of five graded scores of 10 symptoms. We showed that the HSS score, as well as the EQ-5D and VAS scores, decreased after exchanging ARBs for the combination pill. In addition, the change in HSS score correlated significantly with the decrease in systolic blood pressure. Taken together, these results indicate that lowering blood pressure may improve QOL.

We also evaluated sleep quality with the PSQI during the course of treatment with the combination pill. This measurement showed that blood pressure lowering was associated with improved sleep quality, as shown by a decrease in PSQI. Sleep quality is one of the most important factors contributing to QOL. It has been reported that the prevalence of hypertension in subjects who are 'poor sleepers' is 87.1% compared with 35.1% in 'good sleepers'.[24] The increasing interest in the association between sleep disorders and significant comorbidities, including hypertension and glucose metabolism disorders, suggests that studies screening for cardiovascular risk should include an evaluation of sleep quality with questionnaires such as the PSQI. In this regard, our results indicate that the reduction in blood pressure caused by combined losartan /HCTZ treatment may lead to improvements in cardiovascular mortality and morbidity, partially as a consequence of improved sleep quality.

The results of our study also showed that the levels of blood urea nitrogen, uric acid and creatinine increased, whereas eGFR decreased during the 3 months of observation. These results may have been caused by transient decreases in intraglomerular pressure and a subsequent reduction in glomerular filtration, due to the blood pressure-lowering effect of the losartan/HCTZ combination pill. The changes observed in these renal function parameters, however, were within the normal range. In addition, decreases in eGFR were observed only in patients with a normal eGFR ($\geqslant 50\,ml\,min^{-1}\ 1.73\,m^{-2}$), whereas eGFR remained unchanged in patients with a low eGFR ($<50\,ml\,min^{-1}\,1.73\,m^{-2}$). These findings indicate that there are no safety problems associated with the use of the losartan/HCTZ combination pill. Simultaneous decreases in chloride and potassium levels, possibly caused by the effects of HCTZ, were also within the normal range. In this study, blood examination revealed increases in the plasma uric acid levels after 3 months. This increase might have been induced by the administration of HCTZ.

Potential limitations/clinical implications

The major limitation of the S-CATS study was an uncontrolled design involving a single-arm treatment in a relatively small number of patients. It is possible that confounding factors affected the present results. Therefore, we need to perform a two-armed randomized study in the future.

Our study included a limited number of patients because we could not obtain the informed consent from all the patients based on the QOL assessment. However, only practitioners of the Saga Medical Association performed this study and similar efforts by practitioners worldwide would be expected to result in improvements in cardiovascular morbidity and mortality among patients with hypertension. We therefore consider it essential that practitioners who are not specialists in the treatment of hypertension recognize the importance of adequately relieving patients' symptoms, thereby improving QOL and maintaining treatment compliance and medication adherence. On the basis of our results, we envisage that antihypertensive treatment with the losartan/HCTZ combination pill may result in a better long-term prognosis for patients with hypertension, partially as a consequence of improved QOL. In addition, we propose that the evaluation of QOL, including sleep quality, would be useful in the management of hypertension.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Turnbull F. Effects of different blood pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. *Lancet* 2003; **362**: 1527–1535.
2 Turnbull F, Neal B, Pfeffer M, Kostis J, Algert C, Woodward M, Chalmers J, Zanchetti A, MacMahon S. Blood pressure-dependent and independent effects of agents that inhibit the rennin-angiotensin system. *J Hypertens* 2007; **25**: 951–958.
3 Garfield FB, Caro JJ. Compliance and hypertension. *Curr Hypertens Rep* 1999; **1**: 502–506.
4 Haynes RB, McDonald HP, Garg AX. Helping patients follow prescribed treatment: clinical applications. *JAMA* 2002; **288**: 2880–2883.
5 Triverdi RB, Ayotte B, Edrlman D, Bosworth HB. The association of emortional well-being and marital status with treatment adherence among patients with hypertension. *J Behav Med* 2008; **31**: 489–497.
6 DiMatteo MR, Leppper HS, Croghan TW. Depression is a risk factor for noncompliance with medical treatment: meta-analysis of the effects of anxiety and depression on patient adherence. *Arch Intern Med* 2000; **160**: 2101–2107.
7 Brooks R. EuroQol: the current state of play. *Health Policy* 1996; **37**: 53–72.
8 Lamers L, McDonnell J, Stalmeier P, Krabbe PFM, van JB. The Dutch tariff: results and arguments for an effective design for national EQ-5D valuation studies. *Health Econ* 2006; **15**: 1121–1132.
9 Dolan P. Modeling valuations for EuroQol health states. *Medical Care* 1997; **35**: 1095–1108.
10 Brazier J, Deverill M, Green C, Harper R, Booth A. A review of the use of health status measures in economic evaluation. *Health Technol Assess* 1999; **3**: 1–164.
11 Parkin D, Rice N, Lacoby A, Doughty J. Use of a visual analogue scale in a daily patient diary: modelling cross-sectional time-series data on health-related quality of life. *Soc Sci Med* 2004; **54**: 351–360.
12 Buysse DJ, Reynolds III CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* 1989; **28**: 193–213.
13 Carpenter JS, Andrykowski A. Psychometric evaluation of the Pittsburgh Sleep Quality Index. *J Psychosom Res* 1998; **45**: 5–13.
14 Kullman S, Svardsudd K. Differences in perceived symptoms/quality of life in untreated hypertensive and normotensive men. *Scand J Prim Health Care* 1990; **1**: 47–53.
15 Battersby C, Hartley K, Fletcher AF, Markowe HJ, Styles W, Sapper H, Bulpitt CJ. Quality of life in treated hypertension: a case-control community based study. *J Hum Hypertens* 1995; **9**: 981–986.
16 Schoenberger JA, Croog SH, Sudilovsky A, Levine S, Baume RM. Self-reported side effects from antihypertensive drugs. A clinical trial. Quality of Life Research Group. *Am J Hypertens* 1990; **3**: 123–132.