Original Article

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

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A calcium channel blocker (CCB), azelnidipine (AZ), is reported to inhibit oxidative stresses, particularly when administered under blockade of the renin-angiotensin system (RAS). The purpose of this study was to investigate whether AZ inhibits oxidative stresses more potently than other CCBs under blockade of RAS and exerts renoprotection in type 2 diabetic nephropathy. Subjects were hypertensive type 2 diabetics with nephropathy, taking RAS inhibitors. The patients were randomly assigned to two groups, an AZ group (*n*=21, 16 mg/d) and a nifedipine-CR (NF) group (*n*=17, 40 mg/d). The plasma levels of monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), high-sensitive C-reactive protein (hsCRP), adiponectin and tumor necrosis factor-α (TNF_α), the urinary excretion of 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) and 8-hydroxydeoxyguanosine (8-OHdG), and the urinary albumin-to-creatinine ratios (ACR) were determined before and after 16-week treatment. Neither metabolic parameters nor blood pressure levels differed between the two groups not only at baseline but also after the treatment. However, significant decreases in MCP-1, IL-6, hsCRP, TNF_α, 8-epi-$PGF_{2\alpha}$, 8-OHdG and ACR levels, and a significant increase in the plasma adiponectin level were detected in the AZ group, but not in the NF group. The % change in the urinary oxidative stress markers correlated with that in ACR. Our results indicate that, in hypertensive patients with diabetic nephropathy, a combination therapy of RAS inhibitors and AZ is an effective therapeutic modality for decreasing not only blood pressure but also inflammations and oxidative stresses. (*Hypertens Res* 2008; 31: 1147–1155)

***Key Words*: azelnidipine, albuminuria, diabetic nephropathy, oxidative stress, inflammation**

Introduction

Inflammation and oxidative stresses are known to play important roles in the pathogenesis of diabetic and/or hypertensive organ damages (*1*). For example, when advanced glycation end-products (AGEs), shear stresses, and angiotensin II (Ang II) stimulate their respective receptors, NADPH oxidase activity is enhanced and production of oxidative stress is increased (*1*, *2*). It has been proposed that the increased oxidative stress activates many inflammatory cytokines and chemokines, and they elicit various organ damages (*3*). The overproduction of oxidative stress induced by increased Ang II is considered one of the pathophysiological features of diabetic nephropathy. In order to protect against the progression of organ damages in hypertensive patients with diabetes it is important to inhibit Ang II activities, in addition to normalizing the blood glucose level and blood pressure (BP). In fact,

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many studies have demonstrated that renin-angiotensin system (RAS) inhibitors suppress the progression of the diabetic complications (*4*). Oxidative stress is reduced by blockade of Ang II and renal protection is exerted in proportion to the reduction of oxidative stress (*5*). However, since oxidative stress is induced not only by Ang II but also by some other pathways, overproduction of oxidative stress cannot be suppressed by RAS inhibition alone. Thus agents with mechanisms different from that of RAS inhibitors are needed for further renal protection in hypertensive patients with diabetes.

Azelnidipine (AZ) is a new dihydropyridine L-type calcium channel blocker (CCB) that does not cause reflex tachycardia associated with BP reduction (*6*, *7*). In addition to inhibiting Ca^{2+} channels, AZ is reported to have an anti-oxidant effect at the clinically relevant concentrations (*8*). AZ is also reported to suppress oxidative stress in endothelial, mesangial and vascular smooth muscle cells (*9–12*). AZ has been shown to inhibit oxidative stress induced by RAS (*10*). However, AZ may also reduce oxidative stress induced by some other pathway, because its effects were still observed in angiotensin II type 1 (AT1) receptor knock-out mice (*11*). Furthermore, combined administration of AZ with a RAS inhibitor elicited more potent renal protective activity than either agent alone in hypertensive rats complicated with heart failure (*13*). From these results, concomitant use of AZ plus a RAS inhibitor is expected to be more effective than use of either agent alone. In particular, this combination would be expected to have good therapeutic efficacy against diabetic nephropathy. However, there has been a dearth of clinical studies examining whether AZ exerts anti-oxidant and anti-inflammatory effects. In particular, whether concomitant use of a RAS inhibitor and AZ inhibits oxidative stress, elicits anti-inflammatory effects, or clinically exerts renal protective actions has not been investigated in patients with diabetic nephropathy. If AZ exerts an antioxidant effect in humans, AZ under blockade of RAS is expected to exhibit significantly better efficacy than other CCBs in reducing oxidative stress and inflammation. In the present study, the effects of AZ were compared with those of another CCB, nifedipine CR (NF), on oxidative stresses and inflammatory responses in diabetic and hypertensive patients.

Methods

This study is a prospective randomized control trial. The entry period of this study is 1 year. The subjects enrolled in the present study were hypertensive type 2 diabetic out-patients with diabetic nephropathy, who visited our hospitals and fulfilled one or more of the following criteria: mild or moderate hypertension, defined as an office systolic blood pressure (SBP) of 130–199 mmHg and/or diastolic blood pressure (DBP) of 80–110 mmHg. In cases in which the office SBP was 180–199 mmHg, it was also required that the home SBP be 130–180 mmHg. Use of a RAS inhibitor, such as an angiotensin-converting enzyme inhibitor (ACEI) or an angiotensin II type-1 receptor blocker (ARB), for at least 1 year.

1) An HbA1c level less than 8% at the time of enrollment, and for at least 6 months prior.

2) A urinary albumin-to-creatinine ratio (ACR) higher than 30 (μg/mg creatinine [Cr]) (stage of diabetic nephropathy: stage II and higher).

3) A serum Cr level less than 1.5 (mg/dL) and absence of hematuria.

4) Absence of severe diabetic complications such as retinal hemorrhage, neuropathy, and so on.

5) Absence of severe hepatic damages and cerebrovascular disorders.

The subjects were randomly assigned to either the AZ group or NF group, and patients in the AZ group were treated with either azelnidipine (16 mg/d) or nifedipine CR (40 mg/d). BP, body weight, ACR, the plasma levels of monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), adiponectin, tumor necrosis factor-α (TNF_α), high-sensitive C-reactive protein (hsCRP) and HbA1c, serum lipids, and urinary excretions of 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) and 8-hydroxydeoxyguanosine (8-OHdG) were determined before (baseline levels) and after the treatment for 16 weeks (16-week). The antihypertensive agents (AZ and NF) were taken after breakfast and before going to bed. We collected fasting blood and first urine samples in early morning.

MCP-1 is an inflammatory chemokine, while IL-6 is an inflammatory cytokine. Both of them are considered to be deeply involved in diabetic organ damages. On the other hand, 8-epi-$PGF_{2\alpha}$ and 8-OHdG are products of the oxidizing modification of arachidonic acid and DNA, respectively, and they are considered useful as markers of oxidative stresses. The present study was conducted after obtaining informed consent from all subjects, and the study protocol was approved by the ethics committees of Tohoku University Hospital.

Office BP was measured after 5 min in a state of rest at each visit. Home morning BP was measured at every morning in the seated position prior to breakfast within 1 h after wake-up, using an automatic arm-cuff device (HEM401C; Omron Healthcare Co., Kyoto, Japan). Similarly, home night BP was measured every night at bedtime.

The estimated glomerular filtration rate (eGFR) was calculated using the formula recommended by Imai *et al*. (*14*).

Measurements

Plasma levels of MCP-1, IL-6, adiponectin and TNF_α were determined using an MCP-1 ELISA kit (R&D Systems, Minneapolis, USA), human IL-6 ELISA kit (R&D Systems), human adiponectin ELISA kit (Otsuka Pharmaceutical Company, Tokushima, Japan) and human TNF_α Chemiluminescent Immunoassay kit (R&D Systems), respectively. Urinary levels of 8-epi-$PGF_{2\alpha}$ and 8-OHdG were determined using an 8-isoprostane EIA kit (Cayman Chemical Co., Ann Arbor, USA) and enzyme-linked immunosorbent assay kit (Japan

Table 1. Baseline Characteristics of Study Subjects

	Group		*p*
	Azelnidipine	Nifedipine CR	
n	21	17	
Sex (male/female)	11/10	9/8	n.s.
Age (years)	61.7±2.5	59.4±2.4	n.s.
Diabetic duration (years)	12.1±1.7	9.9±1.5	n.s.
Body mass index (kg/m^2)	24.3±0.6	23.7±0.6	n.s.
HbA1c (%)	6.7±0.1	6.6±0.1	n.s.
Systolic blood pressure (mmHg)	159.0±3.9	154.0±3.2	n.s.
Diastolic blood pressure (mmHg)	77.5±2.7	79.4±2.8	n.s.
Creatinine (mg/dL)	0.77±0.05	0.77±0.06	n.s.
Estimated glomerular filtration rate (mL/min)	69.4±6.2	70.2±6.7	n.s.
Total cholesterol (mg/dL)	199.0±8.1	201.0±8.3	n.s.
Triglyceride (mg/dL)	120.0±10.2	128.0±11.0	n.s.
High density lipoprotein-cholesterol (mg/dL)	49.6±2.3	52.6±2.1	n.s.
Atrial natriuretic peptide (pg/mL)	36.7±5.6	33.2±5.3	n.s.
Brain natriuretic peptide (pg/mL)	64.5±8.9	43.8±12.4	n.s.
Ankle brachial index	1.04±0.02	1.03±0.02	n.s.
Pulse wave velocity (cm/s)	1,775±49	1,784±48	n.s.
Max intima-media thickness (mm)	1.58±0.20	1.49±0.18	n.s.
Diabetic retinopathy	14	10	n.s.
Smorker (current/former)	1/5	2/4	n.s.
Oral hypoglycemic agent/insulin	16/15	11/10	n.s.
Statin/aspirin	9/10	7/9	n.s.
Metabolic syndrome	10	7	n.s.

Mean±SEM.

Institute for Control of Aging, Fukuroi, Japan), respectively, and the values were corrected with the urinary level of creatinine.

Statistical Analysis

All statistical analyses were made using Statview 5.0 software (SAS Institute, Cary, USA).

The study sample size of 40 patients provided 80% power at a probability level of 0.05 to detect a 25% difference between each pair of group in the % change in urinary ACR from baseline to 16 weeks (assuming a standard deviation for % changes in ACR of 30%).

All normally distributed data were expressed as the mean±SEM, and their values were statistically analyzed between the groups as well as between the baseline levels and the levels after the treatment within the same groups using paired or unpaired Student's *t*-test. Data that did not show a normal distribution (ACR, 8-epi-PGF$_{2\alpha}$, 8-OHdG, MCP-1, IL-6, TNF$_\alpha$ adiponectin and hsCRP levels) were expressed as the median (range), and the difference between values before and after the treatment within the same group were analyzed using Wilcoxon signed-rank test, while those between the groups were analyzed using Mann-Whitney *U*-test. Since these data showed a normal distribution after logarithmic conversion, their logarithmically converted (Log) values were expressed as the mean±SEM and their values were analyzed using Student's *t*-test. The rates of smoking, insulin treatment and administrations of any medicines were tested using the χ^2-test. The comparison of complication rates of diabetic retinopathy or metabolic syndrome were analyzed with the χ^2-test.

Correlations were determined by the Spearman rank correlation test. Values of $p<0.05$ were considered statistically significant.

Results

A total of 43 patients were enrolled. After acquiring agreement, two of these patients withdrew agreement from the study. In the NF group, 3 patients were omitted due to dizziness and palpitations. None of the administered medicines were changed during the study period.

The clinical backgrounds of the subjects in each group are summarized in Table 1. There were no significant differences in the clinical backgrounds between the AZ group and the NF group. An ACEI was used for 12 patients (temocapril=4, imidapril=4, enalapril=4,) and an ARB for 19 patients (losartan=2, candesartan=5, valsartan=5, telmisartan=3, olmesartan=4) in the AZ group (ARB+ACEI combina-

Table 2. Changes in Plasma and Serum Levels of Inflammatory Cytokines and Chemokines

	Azelnidipine			Nifedipine CR			
	Before	After	p_1	Before	After	p_2	p_3
MCP-1 (pg/mL)							
Median (range)	182 (128–392)	145 (95–317)	*	195 (127–392)	145 (81–355)	n.s.	
Log value	2.29±0.03	2.20±0.03	*	2.31±0.03	2.28±0.04	n.s.	
Δ change	−41.6±16.4			−8.2±17.3			†
% change	−13.8±7.0			−0.3±8.2			†
IL-6 (pg/mL)							
Median (range)	1.0 (0.2–3.5)	0.7 (0.2–3.2)	*	1.0 (0.3–4.0)	1.1 (0.2–4.2)	n.s.	
Log value	−0.16±0.06	−0.38±0.06	*	0.03±0.07	0.02±0.08	n.s.	
Δ change	−0.25±0.09			0.05±0.21			†
% change	−13.7±10.2			23.1±19.8			†
hsCRP (mg/dL)							
Median (range)	0.12 (0.02–0.42)	0.12 (0.03–0.27)	*	0.08 (0.01–0.44)	0.11 (0.01–0.55)	n.s.	
Log value	−1.68±0.07	−2.09±0.06	*	−1.08±0.11	−1.10±0.13	n.s.	
Δ change	−0.05±0.02			0.01±0.03			†
% change	−8.2±11.9			41.2±25.8			†
Adpn (μg/mL)							
Median (range)	8.8 (2.4–13.9)	8.8 (4.1–24.6)	*	14.0 (3.2–49.1)	11.6 (2.2–38.7)	n.s.	
Log value	0.87±0.05	0.99±0.05	*	1.03±0.08	1.02±0.08	n.s.	
Δ change	2.93±1.10			−0.67±1.49			†
% change	43.3±13.3			7.21±12.3			†
TNF_α (pg/mL)							
Median (range)	2.51 (1.16–5.13)	1.67 (1.00–3.79)	*	1.88 (1.03–5.51)	1.77 (1.06–5.45)	n.s.	
Log value	0.38±0.04	0.25±0.04	*	0.28±0.05	0.32±0.05	n.s.	
Δ change	−0.68±0.16			0.21±0.18			†
% change	−21.6±6.0			16.8±11.5			†

p_1 and p_2: before *vs*. after, *$p<0.01$; p_3: AZ *vs*. NF, †$p<0.01$. Mean±SEM. MCP-1, monocyte chemoattractant protein-1; IL-6, inteleukin-6; hsCRP, high-sensitive C-reactive protein; Adpn, adiponectin; TNF_α, tumor necrosis factor-α; AZ, azelnidipine; NF, nifedipine CR.

Table 3. Changes in Urinary Excretion of Albumin and Oxidative Stress Markers

	Azelnidipine			Nifedipine CR			
	Before	After	p_1	Before	After	p_2	p_3
ACR (μg/mg Cr)							
Median (range)	217 (70–1,783)	244 (35–1,125)		340 (44–1,990)	261 (54–1,167)	n.s.	
Log value	2.49±0.08	2.32±0.09	*	2.53±0.11	2.47±0.09	n.s.	
Δ change	−157.8±61.2			−132.7±68.1			†
% change	−16.7±10.9			18.1±31.7			†
8-epi-$PGF_{2\alpha}$ (pg/mg Cr)							
Median (range)	366 (147–812)	239 (102–566)	*	277 (101–756)	289 (142–548)	n.s	
Log value	2.54±0.05	2.37±0.05	*	2.44±0.06	2.46±0.05	n.s.	
Δ change	−127.1±38.0			1.7±44.4			†
% change	−24.2±8.1			19.8±15.2			†
8-OHdG (ng/mg Cr)							
Median (range)	8.0 (4.1–16.1)	7.1 (4.8–11.5)	*	8.5 (1.8–29.6)	9.7 (2.8–20.2)	n.s.	
Log value	0.91±0.04	0.85±0.02	*	0.95±0.07	0.96±0.06	n.s.	
Δ change	−1.69±0.73			−0.21±0.92			†
% change	−5.91±9.93			10.27±10.41			†

p_1 and p_2: before *vs*. after, *$p<0.01$; p_3: AZ *vs*. NF, †$p<0.01$. Mean±SEM. ACR, urinary albumin-to-creatinine ratio; Cr, creatinine; 8-epi-$PGF_{2\alpha}$, 8-epi-prostaglandin $F_{2\alpha}$; 8-OHdG, 8-hydroxydeoxyguanosine; AZ, azelnidipine; NF, nifedipine CR.

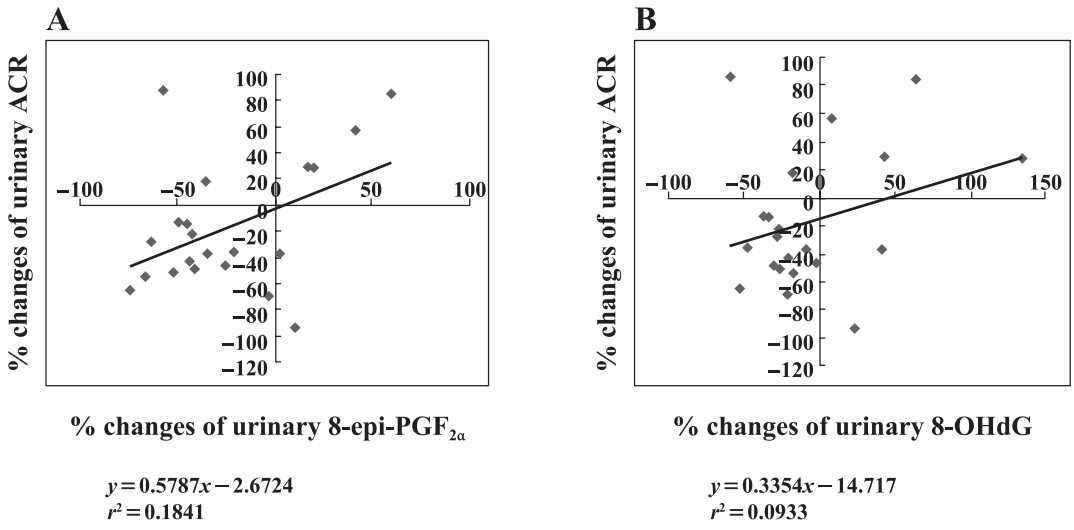

Fig. 1. *The relationship between the % change of urinary 8-epi-PGF$_{2\alpha}$ (A) and 8-OHdG (B) and % change of ACR in the azelnidipine group.*

tion=10), while 9 and 14 patients were treated with an ACEI (temocapril=2, imidapril=4, enalapril=3) and an ARB (losartan=1, candesartan=4, valsartan=3, telmisartan=2, olmesartan=4), respectively, in the NF group (ARB+ACEI combination=7). No CCBs or diuretics were administered. Statins were administered for 9 and 7 patients in the AZ and the NF groups, respectively. Aspirin was taken by 10 and 9 patients in the AZ and the NF groups, respectively. Pioglitazone was administered to 7 and 5 patients in the AZ and the NF groups, respectively. There were 10 and 7 patients who met the criteria for metabolic syndrome in the AZ and the NF groups, respectively (Table 1).

Both office and home morning BP (SBP/DBP, mean±SEM) were significantly lowered following the treatment in both the AZ group (office: from 158.8±3.89/78.9±1.06 to 144.4±4.62/77.7±1.03 mmHg; home morning: from 158.4±2.61/81.6±1.14 to 139.9±2.91/78.9±0.94 mmHg) and NF groups (office: from 154.2±3.22/79.5±1.12 to 142.5±5.34/77.9±1.01 mmHg; home morning: from 153.5±3.48/81.9±1.13 to 140.3±4.98/79.0±0.98 mmHg). The baseline levels and the changes in BP were not significantly different between the two groups. HbA1c, serum triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) levels were not altered following the treatment with AZ or NF, and these levels in the AZ group were not significantly different from those in the NF group. Thus clinical background characteristics, including changes in BP, and glycemic and lipid control, were equivalent between the AZ and the NF groups. Although, diabetic retinopathy is not identified in several patients (7 in the AZ group, 7 in the NF group). these patients were all within the levels of microalbuminuria.

Table 2 shows the changes in plasma MCP-1, IL-6, hsCRP, adiponectin and TNF$_\alpha$ levels in the AZ and the NF groups. These values represent the median value (range), logarithmic converted value, the difference between observed values before and after treatment, and the rate of change (% change) before and after the treatment. In the AZ group, the levels of MCP-1, IL-6, hsCRP, and TNF$_\alpha$ were significantly decreased, whereas adiponectin was significantly increased at the end of the treatment. Furthermore, the difference and the % change of these biomarkers induced by AZ treatment were significantly greater than those induced by NF.

Table 3 shows the changes in urinary 8-epi-PGF$_{2\alpha}$, 8-OHdG and ACR in the AZ and the NF groups. These values represent the median value (range), logarithmic converted value, differences in values before and after treatment, and % change from the baseline to the end of the study. Levels of 8-epi-PGF$_{2\alpha}$, 8-OHdG, and ACR in the AZ group were significantly decreased after the treatment compared with beforehand. Furthermore, the absolute (Δ) and % changes induced by the treatment were significantly larger in the AZ group as compared with the NF group.

Figure 1 illustrates the relationship between the % change in urinary 8-epi-PGF$_{2\alpha}$ (A) and 8-OHdG (B) and the % change of ACR in the AZ group. As can be seen, the % change in the urinary oxidative stress markers was correlated with that in ACR although the correlation was weak.

Figure 2 shows the correlation between the % change in BP and that of ACR. There were significant relationships between the % change in ACR and that in SBP in both the AZ and NF groups. In addition, the correlation coefficient was better with home morning SBP compared with office SBP. There was no significant relationship between the % change in ACR and that in home night SBP. Moreover, with the same degree of SBP reduction, decreases in ACR in the AZ group

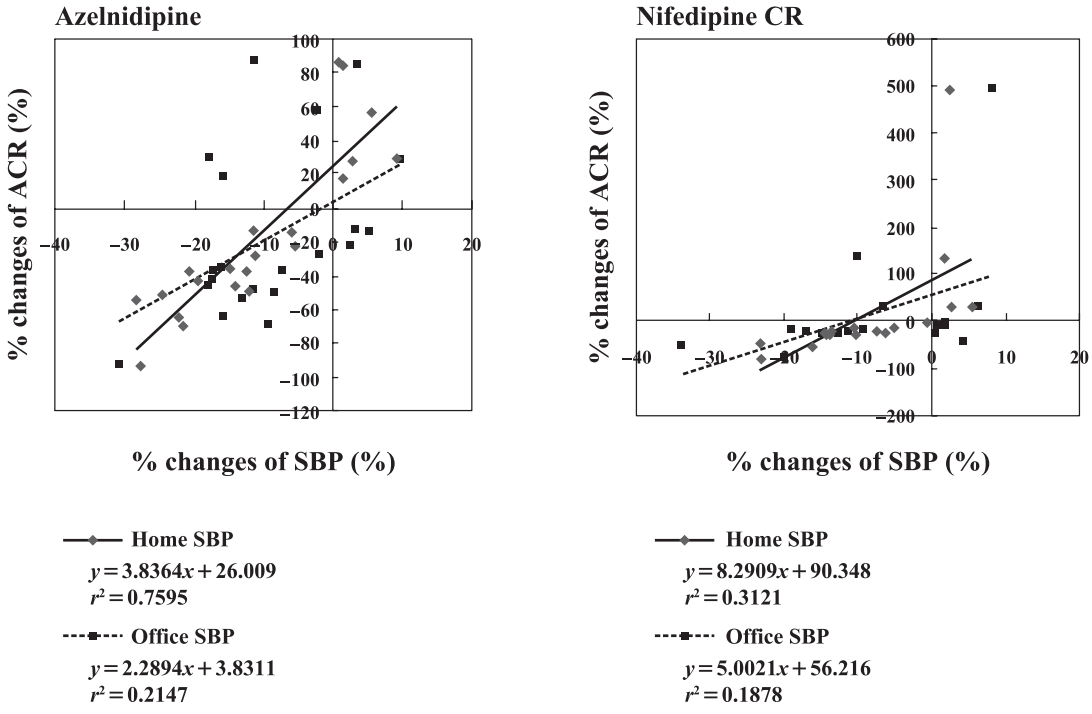

Fig. 2. *The correlation between the % change of blood pressure and that of ACR.*

were greater than those in the NF group.

The type of RAS inhibitors did not affect the results of this study. There were no differences in the changes of these markers with or without statins, aspirin and pioglitazone. The presence of metabolic syndrome did not influence the results of this study.

Although there is no heart failure sign on cardiac echogram (decrease of ejection fraction, cardiac hypertrophy, *etc*.), the plasma levels of brain natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) were slightly high at baseline. However, there was no subject who developed cardiac failure in this study. Moreover, significant changes of BNP and ANP levels were not observed between before and after the treatment.

Discussion

In hypertension with diabetic nephropathy, various factors, including Ang II, enhance oxidative stress through the activation of NADPH oxidase. Oxidative stress produced in this manner potently promotes renal damages. Inhibition of oxidative stress by RAS inhibition is important for renal protection (*5*). However, since oxidative stress is not induced by RAS alone in patients with diabetic nephropathy, overproduction of oxidative stress cannot be suppressed sufficiently by inhibiting RAS. Indeed, there are many patients in whom sufficient renal protection is not attained even by treatment with RAS inhibitors. Thus agents with mechanisms different from that of RAS inhibitors are needed to further protect the kidney.

AZ is reported to suppress oxidative stress induced by RAS (*10*). AZ may also suppress oxidative stress and inflammatory reactions induced by some other pathways, because it has been reported that, in AT1 receptor–knock out mice (*11*), AZ ameliorated vascular injuries induced by a cuff, while it inhibited superoxide production, expression of NADPH oxidase subunits, and TNF_{α}-induced MCP-1 and IL-8 production (*9*, *15–17*). In addition, it has been reported that AZ and RAS inhibition exert synergistic actions in both reducing oxidative stress and ameliorating tissue damages in models of stroke and atherosclerosis (*17*, *18*). Moreover, AZ is reported to prevent renal damage caused by AGE (*19*). From these results, concomitant use of AZ and RAS inhibitors is expected to be more effective than use of either agent alone. However, there has been no clinical investigation clarifying the usefulness of this combination therapy in terms of anti-albuminuric or antihypertensive effects in diabetic nephropathy, nor has there been any study examining the anti-oxidative and anti-inflammatory effects of AZ and their relations to anti-albuminuric action.

In the present study, we observed that in the patients already treated with RAS inhibitors, AZ decreased ACR, plasma levels of inflammatory markers, and urinary excretions of oxidative stress markers. In contrast, NF had no such effects even though BP was decreased to a similar degree in both the AZ and NF group. AZ and NF belong to the family of dihydropyridine-type CCBs, which block L-type calcium channels. Thus, the effects of AZ we observed may not be explained merely by the reduction of BP or inhibition of L-

type calcium channels. Since all the patients had already been treated with RAS inhibitors, our results may also indicate that AZ may suppress oxidative stress and inflammatory responses independently of the RAS, or that it may synergistically or additively act with RAS blockade. However, we could not distinguish these two possibilities, since treating diabetic nephropathy patients without RAS inhibition would be deemed unethical. RAS inhibitors are mandatory in the treatment of diabetic nephropathy. Our results suggest that AZ may be an additional effective therapeutic modality to retard or inhibit the progression of diabetic nephropathy.

The mechanisms by which AZ reduces urinary and plasma markers of oxidative stress and inflammation are not clear from the present study. AZ is a highly lipophylic CCB that has a high tissue affinity, and an anti-oxidant property that is based on its chemical structure (*20*). It has been shown that at concentrations seen in the plasma of patients taking daily clinical doses of AZ (1–10 nmol/L), AZ inhibits hydrogen peroxide–induced cell injuries in cultured human endothelial cells (*8*). And AZ inhibited the H_2O_2-induced c-Jun NH2-terminal kinase (JNK) activation (JNK accelerates apoptosis) and cell death in neonatal rat cardiomyocytes (*21*). These actions may be related to AZ's direct scavenging of hydrogen radicals within the cells. In addition, AZ has been shown to suppress the NADPH oxidase expression and activity induced by various stimul (*9*, *11*, *12*, *15–17*, *22*).

Adiponectin is an anti-atherosclerotic substance produced by adipose tissues. It has been shown that adiponectin production is suppressed by stimulation of AT1 receptors and/or heightened oxidative stress (*23*, *24*). Studies have also revealed that macrophages infiltrating into adipose tissues play significant roles in regulating the production of adipocytokines, such as TNF_α, IL-6 and adiponectin (*25–27*). It is well established that RAS inhibition increases plasma adiponectin levels in humans, and this effect is closely related to decreased levels of oxidative stress and inflammatory markers (*23*). However, the issue of whether CCBs increase plasma adiponectin levels has not been fully investigated. Some studies have reported that CCBs increase plasma adiponectin levels, while other studies have observed no such effects (*28–31*). Such divergent results may be due to differences in the properties of individual CCBs, the diseases of subjects studied and/or the background treatments. It has been reported that plasma adiponectin levels are associated with the arterial BP, body fat content and lipid parameters in hypertensive patients with metabolic syndrome (*31*). In the present study, the plasma adiponectin level was increased by AZ but not NF in diabetic nephropathy patients who had previously been treated with RAS inhibitors. A previous study reported that NF increased plasma adiponectin levels in diabetic patients who were not treated with RAS inhibitors (*30*). Thus, the reported ability of NF to increase adiponectin levels may have been mediated by the same pathway by which RAS inhibition increases adiponectin. On the other hand, AZ may have some distinct mechanisms for increasing adiponectin. Since AZ has been reported to reduce MCP-1 (*11*, *15*), it may have inhibited macrophage activation, resulting in an increase in adiponectin and decrease in inflammatory cytokines (*32–34*).

While animal studies have reported renoprotective effects AZ (*35*, *36*), there has been only one clinical study that has examined the renoprotective action of AZ. Nakamura *et al*. recently reported that AZ reduced urinary protein excretion and the urinary levels of 8-OHdG and liver-type fatty acid binding protein (a clinical biomarker of tubulointerstitial damage) in hypertensive patients with chronic kidney diseases (CKD) (*7*). These data suggest that AZ may ameliorate renal injuries in part by reducing oxidative stress within the tubulointerstitium. Recent studies have indicated that derangement of peritubular capillary circulation with consequent tubulointerstitial hypoxia plays a pivotal role in the pathogenesis of renal injury (*37*). It has been reported that AZ attenuates Ang II–induced peritubular ischemia, mitochondrial injury and apoptosis in hypoxic renal tubular cells (*37*, *38*). In the study of Nakamura *et al*. (*7*), however, only a few subjects were treated with RAS inhibitors. In the present study, we provide the first clinical evidence that AZ confers renoprotection in diabetic nephropathy. Namely, AZ was found to decrease ACR and urinary markers of oxidative stress, and there were significant relationships between them. It is of note that all of our patients had already been treated with RAS inhibitors.

Reducing BP is known to decrease ACR (*39*). In the present study, greater reductions of BP were associated with greater decreases in ACR in both the AZ and NF group. However, the slope was much steeper in the AZ than the NF group, such that, at the same degree of BP reduction, AZ decreased ACR more than NF. This suggests that AZ may have some mechanisms for reducing ACR other than merely blocking L-type calcium or decreasing BP. Our study also showed that changes in ACR were more closely related with changes in home SBP at wake-up than changes in office BP. In patients with diabetes mellitus, nocturnal hypertension caused by enhanced activity of the sympathetic nervous system (SNS) may contribute to renal damage (*40*, *41*). Thus, the finding that ACR has a closer relationship with home wake-up BP may be related to the nocturnal BP imposing a greater burden on kidneys. It has been suggested that CCBs may activate the SNS and increase heart rates because of their potent hypotensive action (*42*, *43*). Unlike other dihydropyridine-type CCBs, AZ has a unique action of inhibiting SNS, and it indeed decreases, rather than increases, the heart rate after oral administration (*6*, *44*). Antihypertensive treatment with AZ attenuates reflex-induced sympathetic activation and enhances endothelial nitric oxide synthase expression levels in the brain as well as in the heart and aorta (*45*). In addition to its anti-oxidant activity, AZ's inhibitory action on the SNS may have contributed to a greater anti-albuminuric effect as compared with NF.

Limitations of the Trial

This is a clinical study involving human subjects, and thus there was a limit to its elucidation of the mechanism by which AZ reduced ACR and oxidative stress. The issue of whether our results can be extended to patients with diabetic nephropathy in general awaits further investigation with larger numbers of patients.

Potential Clinical Implications

Administration of a RAS inhibitor is one of the currently available therapeutic options for hypertensive patients with diabetic nephropathy. However, this treatment alone is insufficient in many cases. While CCBs are widely used to treat hypertension, the issue of whether CCBs can exert renoprotective effects beyond their BP-lowering actions remains controversial. As shown in the present study, all CCBs are not the same. In addition to blocking L-type calcium channels, the newly developed dihydropyridine CCBs have some additional properties, such as anti-oxidant and SNS-inhibitory actions, as seen in the case of AZ, or blockade of the T-type calcium channels (efonidipine) or N-type channels (cilnidipine). These characteristics should be taken into consideration when selecting CCBs for individual patients with different clinical features. Clearly, further studies are needed to clarify these issues.

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References

1. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001; **414**: 813–820.
2. Sheetz MJ, King GL: Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *JAMA* 2002; **288**: 2579–2588.
3. Taniyama Y, Griendling KK: Reactive oxygen species in the vasculature: molecular and cellular mechanisms. *Hypertension* 2003; **42**: 1075–1081.
4. 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**: 672–678.
5. Ogawa S, Mori T, Nako K, *et al*: Angiotensin II type 1 receptor blockers reduce urinary oxidative stress markers in hypertensive diabetic nephropathy. *Hypertension* 2006; **47**: 699–705.
6. Yamagishi T: Efficacy of azelnidipine on home blood pressure and pulse rate in patients with essential hypertension: comparison with amlodipine. *Hypertens Res* 2006; **29**: 767–773.
7. Nakamura T, Sugaya T, Kawagoe Y, *et al*: Azeinidipine 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**: 321–326.
8. Shinomiya K, Mizushige K, Fukunaga M, *et al*: Antioxidant effect of a new calcium antagonist, azelnidipine, in cultured human arterial endothelial cells. *J Int Med Res* 2004; **32**: 170–175.
9. Yamagishi S, Inagaki Y, Nakamura K, *et al*: Azelnidipine, a newly developed long-acting calcium antagonist, inhibits tumor necrosis factor-alpha–induced interleukin-8 expression in endothelial cells through its anti-oxidative properties. *J Cardiovasc Pharmacol* 2004; **43**: 724–730.
10. Matsui T, Yamagishi S, Nakamura K, *et al*: Azelnidipine, a dihydropyridine-based calcium antagonist, inhibits angiotensin II–induced oxidative stress generation and down-regulation of pigment epithelium-derived factor mRNA levels in microvascular endothelial cells. *Drugs Exp Clin Res* 2005; **3**: 215–219.
11. Jinno T, Iwai M, Li Z, *et al*: Calcium channel blocker azelnidipine enhances vascular protective effects of AT1 receptor blocker olmesartan. *Hypertension* 2004; **43**: 263–269.
12. Manabe S, Okura T, Fukuoka T, *et al*: Antioxidative effects of azelnidipine on mesangial cell proliferation induced by highly concentrated insulin. *Eur J Pharmacol* 2007: **567**: 252–257.
13. Kim-Mitsuyama S, Izumi Y, Izumiya Y, *et al*: Additive beneficial effects of the combination of a calcium channel blocker and an angiotensin blocker on a hypertensive rat-heart failure model. *Hypertens Res* 2004; **27**: 771–779.
14. Imai E, Horio M, Nitta K, *et al*: Modification of the MDRD Study equation for Japan. *Am J Kidney Dis* 2007; **50**: 927–937.
15. Matsui T, Yamagishi S, Nakamura K, Inoue H: Azelnidipine, a new long-acting calcium-channel blocker, inhibits tumor necrosis factor-α–induced monocyte chemoattractant protein-1 expression in endothelial cells. *J Int Med Res* 2006; **34**: 671–675.
16. Nakamura K, Yamagishi S, Inoue H: Unique atheroprotective property of azelnidipine, a dihydropyridine-based calcium antagonist. *Med Hypotheses* 2005; **65**: 155–157.
17. Suzuki J, Iwai M, Li Z, *et al*: Effect of combination of calcium antagonist, azelnidipine, and AT1 receptor blocker, olmesartan, on atherosclerosis in apolipoprotein E–deficient mice. *J Hypertens* 2005; **23**: 1383–1389.
18. Iwai M, Chen R, Ide A, *et al*: The calcium-channel blocker, azelnidipine, enhances the inhibitory action of AT1 receptor blockade on ischemic brain damage. *J Hypertens* 2006; **24**: 2023–2031.
19. Yamagishi S, Takeuchi M, Inoue H: Renoprotective effects of azelnidipine, a dihydropyridine-based calcium antagonist in advanced glycation end product (AGE)–injected rats. *Int J Tissue React* 2005; **27**: 1337–1343.
20. Koike H, Kimura T, Kawasaki T, *et al*: Azelnidipine, a long-acting calcium channel blocker with slow onset and high vascular affinity. *Annu Rep Sankyo Res Lab* 2002: **54**: 1–64.
21. Koyama Y, Takeishi Y, Takahashi H, *et al*: Azelnidipine inhibits H_2O_2-induced cell death in neonatal rat cardiomyo-

cytes. *Cardiovasc Drugs Ther* 2007; **21**: 69–72.
22. Naito Y, Shimozawa M, Manabe H, *et al*: Azelnidipine, a new calcium channel blocker, inhibits endothelial inflammatory response by reducing intracellular levels of reactive oxygen species. *Eur J Pharmacol* 2006; **546**: 11–18.
23. Kurata A, Nishizawa H, Kihara S, *et al*: Blockade of angiotensin II type-1 receptor reduces oxidative stress in adipose tissue and ameliorates adipocytokine dysregulation. *Kidney Int* 2006; **70**: 1717–1724.
24. Furuhashi M, Ura N, Higashiura K, *et al*: Blockade of the renin-angiotensin system increases adiponectin concentrations in patients with essential hypertension. *Hypertension* 2003; **42**: 76–81.
25. Gustafson B, Hammarstedt A, Andersson CX, *et al*: Inflamed adipose tissue: a culprit underlying the metabolic syndrome and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007; **27**: 2276–2283.
26. Ailhaud G: Adipose tissue as a secretory organ: from adipogenesis to the metabolic syndrome. *C R Biol* 2006; **329**: 570–577.
27. Coenen KR, Gruen ML, Chait A, *et al*: Diet-induced increases in adiposity, but not plasma lipids, promote macrophage infiltration into white adipose tissue. *Diabetes* 2007; **56**: 564–573.
28. Piecha G, Adamczak M, Chudek J, *et al*: Indapamide decreases plasma adiponectin concentration in patients with essential hypertension. *Kidney Blood Press Res* 2007; **30**: 187–194.
29. Watanabe S, Okura T, Kurata M, *et al*: The effect of losartan and amlodipine on serum adiponectin in Japanease adults with essential hypertension. *Clin Ther* 2006; **28**: 1677–1685.
30. Nomura S, Inami N, Kimura Y, *et al*: Effect of nifedipine on adiponectin in hypertensive patients with type 2 diabetes mellitus. *J Hum Hypertens* 2007; **21**: 38–44.
31. Yilmaz MI, Sonmez A, Caglar K, *et al*: Effect of antihypertensive agents on plasma adiponectin levels in hypertensive patients with metabolic syndrome. *Nephrology* (*Carlton*) 2007; **12**: 1447–1453.
32. Kamei N, Tobe K, Suzuki R, *et al*: Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. *J Biol Chem* 2006; **281**: 26602–26614.
33. Kang L, Sebastian BM, Pritchard MT, Pratt BT, Previs SF, Nagy LE: Chronic ethanol-induced insulin resistance is associated with macrophage infiltration into adipose tissue and altered expression of adipocytokines. *Alcohol Clin Exp Res* 2007; **31**: 1581–1588.
34. Woo HM, Kang JH, Kawada T, *et al*: Active spice-derived components can inhibit inflammatory responses of adipose tissue in obesity by suppressing inflammatory actions of macrophages and release of monocyte chemoattractant protein-1 from adipocytes. *Life Sci* 2007; **80**: 926–931.
35. Yagil Y, Miyamoto M, Frasier L, *et al*: Effects of CS-905, a novel dihydropyridine calcium channel blocker, on arterial pressure, renal excretory function, and inner medullary blood flow in the rat. *Am J Hypertens* 1994; **7**: 637–646.
36. Kanazawa M, Kohzuki M, Yoshida K, *et al*: Combination therapy with an angiotensin-converting enzyme (ACE) inhibitor and a calcium antagonist: beyond the renoprotective effects of ACE inhibitor monotherapy in a spontaneous hypertensive rat with renal ablation. *Hypertens Res* 2002; **25**: 447–453.
37. Tanaka T, Nangaku M, Miyata T, *et al*: Blockade of calcium influx through L-type calcium channels attenuates mitochondrial injury and apoptosis in hypoxic renal tubular cells. *J Am Soc Nephrol* 2004; **15**: 2320–2333.
38. Kondo N, Kiyomoto H, Yamamoto T, *et al*: Effects of calcium channel blockade on angiotensin II–induced peritubular ischemia in rats. *J Pharmacol Exp Ther* 2006; **316**: 1047–1052.
39. Ogawa S, Takeuchi K, Mori T, *et al*: Effects of monotherapy of temocapril or candesartan with dose increments or combination therapy with both drugs on the suppression of diabetic nephropathy. *Hypertens Res* 2007; **30**: 325–334.
40. Nielsen FS, Hansen HP, Jacobsen P, *et al*: Increased sympathetic activity during sleep and nocturnal hypertension in Type 2 diabetic patients with diabetic nephropathy. *Diabet Med* 1999; **16**: 555–562.
41. Perin PC, Maule S, Quadri R: Sympathetic nervous system, diabetes, and hypertension. *Clin Exp Hypertens* 2001; **23**: 45–55.
42. Scholz H: Pharmacological aspects of calcium channel blockers. *Cardiovasc Drugs Ther* 1997; **10**: 869–872.
43. Damase-Michel C, Valet P, Montastruc JL: Nicardipine causes sympathetic activation that does not involve baroreceptor reflex tachycardia in conscious sinoaortic-denervated dogs. *Eur J Pharmacol* 1987; **142**: 145–149.
44. 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**: 1017–1023.
45. Kimura Y, Hirooka Y, Sagara Y, *et al*: Long-acting calcium channel blocker, azelnidipine, increases endothelial nitric oxide synthase in the brain and inhibits sympathetic nerve activity. *Clin Exp Hypertens* 2007; **29**: 13–21.