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The effect of spironolactone on circulating adipocytokines in patients with type 2 diabetes mellitus complicated by diabetic nephropathy

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Abstract

Angiotensin II can influence adipocytokine levels in adipose tissue, but the association between aldosterone, which mediates the effect of angiotensin II, and adipocytokines has yet to be fully elucidated. This study was designed to investigate the effect of spironolactone, a representative aldosterone blocker, on adipocytokines such as adiponectin, visfatin, plasminogen activator inhibitor (PAI)-1 and tumor necrosis factor α in patients with type 2 diabetic nephropathy: the study included 33 patients, 22 of whom were randomly assigned to the spironolactone (50 mg/d) group and 11 to the amlodipine (2.5 mg/d) group. Data were collected at baseline and after 3 months of treatment and compared with baseline data for 25 age-matched healthy subjects. A significant decrease in plasminogen activator inhibitor 1 in the spironolactone group was observed (22.6 ± 13.4 to 19.2 ± 11.3 ng/mL, P =.0323), but this did not occur in the amlodipine group. Adiponectin and visfatin levels did not change in the spironolactone and amlodipine groups, but significant increases in these adipocytokines were found in a subgroup of patients in the spironolactone group with glycated hemoglobin A_{1c} (HbA_{1c}) 8.0% or greater (11.8 ± 6.4 to 13.3 ± 7.4 μg/mL, P = .0344; and 1.39 ± 0.92 to 2.26 ± 0.76 ng/mL, P =.0397, respectively). The tumor necrosis factor α level at baseline exceeded the lower detection limit of the assay in only 6 patients in the spironolactone group, and no change occurred in these patients. Moreover, neither spironolactone nor amlodipine therapy caused a change in high-sensitivity C-reactive protein or soluble CD40 ligand, but a significant decrease in the level of brain natriuretic peptide was found in the spironolactone group only. Furthermore, significant increases of HbA_{1c}, creatinine, potassium, and aldosterone levels and plasma renin activity, and a decrease in urinary albumin excretion were also observed only in the spironolactone group. The number of patients with HbA_{1c} 8.0% or greater increased after spironolactone treatment. A significant decrease in systolic but not in diastolic blood pressure was observed in both treatment groups. In conclusion, our data suggest that in patients with type 2 diabetes mellitus complicated by diabetic nephropathy, spironolactone can decrease plasminogen activator inhibitor 1 and brain natriuretic peptide levels in addition to urinary albumin excretion, and systolic blood pressure, and that in patients with poor glycemic control, spironolactone can increase the levels of adiponectin and visfatin. However, the significant elevation of HbA_{1c} levels by spironolactone should be emphasized.

1. Introduction

Adipocytokines such as adiponectin, tumor necrosis factor (TNF) α and plasminogen activator inhibitor 1 (PAI-1) are produced in adipose tissues and have various physiologic activities. These molecules have been associated with cardiovascular events [1-3], insulin resistance [4,5], and the onset of diabetes [6,7], and adiponectin, which is produced specifically in adipose tissue, plays a protective role against atherosclerosis, unlike TNF-α and PAI-1 [8]. Furthermore, a recently identified adipocytokine, visfatin, has a remarkable insulin-like action [9].

The renin-angiotensin system (RAS) is related not only to blood pressure, but also to various organic impairments [10,11] and insulin resistance [12]. It is now apparent that fatty cells express angiotensin II (A-II) type 1 receptor in addition to angiotensinogen [13], suggesting that the RAS may also have a role in adipose tissue and an association with adipocytokines. In fact, clinical studies demonstrate that administration of angiotensin-converting enzyme inhibitor (ACE-I) or A-II receptor blocker (ARB) increases serum adiponectin concentrations [14,15]. However, recent reports also indicate that the organic impairment effects of

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A-II are partially mediated by aldosterone induced by A-II [16,17]. The mineralocorticoid receptor (MR) is also expressed in adipose tissue [18], suggesting that aldosterone, in addition to A-II, might influence the production of adipocytokines in adipose tissue.

The association between aldosterone and adipose tissue or adipocytokines is not fully understood, and the effects of aldosterone blockers on circulating adiponectin or visfatin concentrations have not been investigated. However, it has recently been reported that spironolactone, a representative aldosterone blocker, may have a beneficial effect on diabetic patients, especially in those with diabetic nephropathy [19,20], who are likely to have endothelial dysfunction and an elevated risk of cardiovascular events [21,22].

Given this background, in the current study we explored the effects of spironolactone on adipocytokines, such as adiponectin, visfatin, TNF-α, and PAI-1, and on urinary albumin excretion (UAE) in patients with type 2 diabetes mellitus complicated by diabetic nephropathy. We also investigated the levels of high-sensitivity C-reactive protein (hsCRP), an inflammatory marker; soluble CD40 ligand (sCD40L), a newly discovered predictor of cardiovascular events [23]; and brain natriuretic peptide (BNP), a marker of chronic heart failure (CHF), in these patients. We hypothesized that spironolactone therapy would increase circulating adiponectin and visfatin, but decrease TNF-α and PAI-1, as well as hsCRP, sCD40L, and BNP.

2. Patients and methods

2.1. Patients

Thirty-three patients with type 2 diabetes mellitus with diabetic nephropathy were recruited consecutively from June 2004 to June 2005 in the outpatient department of our hospital. Diabetic nephropathy was defined as having UAE of more than 30 mg/g creatinine (Cr), based on the guidelines of the American Diabetes Association [24]. All patients with Cr exceeding 1.0 mg/dL were excluded. In the spironolactone group, 1 patient had a potassium concentration of 5.0 mmol/L at baseline, but all others had a concentration of less than 5.0 mmol/L. Any patient exhibiting evidence of liver dysfunction or findings of infectious or autoimmune disease was excluded from the study. No patients showed clinically apparent symptoms of CHF; an ultrasonographic evaluation of changes in cardiac function was not performed, but 7 patients in the spironolactone group had undergone a cardiac ultrasonography test during a medical checkup within 6 months before administration of spironolactone and showed only normal findings. To diminish the possible influence of antihypertensive drugs on the RAS, only use of doxazosin (an α_1 adrenal receptor blocker) was permitted as an antihypertensive drug. Four patients in the spironolactone group and 1 in the amlodipine group received doxazosin. During recruitment, patients treated with antihypertensive drugs other than doxazosin were excluded from the study.

Table 1
Clinical characteristics and laboratory data of the age-matched nondiabetic control subjects, the entire diabetic patient group, and the diabetic patients treated with spironolactone

	Control subjects	Diabetic patients	Spironolactone treated[a]	$P_{(1)}$	$P_{(2)}$
No. (male/female)	25 (9/16)	33 (13/20)	22 (8/14)	–	–
Age (y)	62.8 ± 4.9	58.9 ± 10.1	60.1 ± 8.0	.0600	.1798
Duration (y)	–	12.2 ± 9.1	12.9 ± 6.5	–	–
BMI (kg/m^2)	21.4 ± 2.7	25.2 ± 4.9	24.6 ± 4.2	.0033*	.0042*
FPG (mmol/L)	5.44 ± 0.54	8.17 ± 2.80	7.81 ± 2.55	.0004*	.0065*
HbA_{1c} (%)	4.9 ± 0.3	7.6 ± 1.6	7.6 ± 1.4	<.0001*	<.0001*
SBP (mm Hg)	124.0 ± 8.9	136.1 ± 8.7	135.5 ± 8.2	<.0001*	<.0001*
DBP (mm Hg)	73.8 ± 6.7	72.4 ± 5.9	71.9 ± 5.7	04125	.2884
UAE (mg/g Cr)	17.7 (10.6, 38.2)	457 (163, 1114.5)	539 (168.3, 1164.8)	<.0001*	<.0001*
Adiponectin (μg/mL)	12.1 ± 5.1	14.0 ± 7.6	14.3 ± 9.1	.2702	.3386
PAI-1 (ng/mL)	22.9 ± 5.3	25.9 ± 17.1	22.6 ± 13.4	.3492	.9367
Retinopathy (n) (NDR, SDR, PDR)	–	(14/5/14)	(7/4/11)	–	–
Statins (n)	–	9	4	–	–
Therapy (n)					
Diet	–	3	1	–	–
SU1/SU2/SU3/SU4/SU5	–	4/1/4/2/6	3/1/4/0/3	–	–
Voglibose alone	–	1	0	–	–
Insulin	–	14	10	–	–

All data except for UAE are expressed as mean ± SD, but not SE. Comparisons in variables except for UAE between the 2 groups were made by use of an unpaired *t* test. UAE is expressed as median with interquartile range (25th and 75th percentiles). Comparison in UAE between 2 groups were made by use of a Wilcoxon rank sum test. $P_{(1)}$: *P* value for 25 control subjects vs 33 entire diabetic patients. $P_{(2)}$: *P* value for 25 control subjects vs 22 diabetic patients treated with spironolactone.

DBP indicates diastolic blood pressure; NDR, normal diabetic retinopathy; SDR, simple diabetic retinopathy; PDR, proliferative diabetic retinopathy; SU1, number of patients treated with sulfonylurea alone; SU2, number of patients treated with sulfonylurea and pioglitazone; SU3, number of patients treated with sulfonylurea and metformin; SU4, number of patients treated with sulfonylurea and voglibose; SU5, number of patients treated with sulfonylurea, metformin, and voglibose.

[a] Diabetic patients treated with spironolactone (50 mg/d).

* $P < .05$, statistically significant.

Twenty-five age-matched healthy subjects were recruited as controls, and the characteristics and laboratory data of these 25 controls and of the 33 diabetic patients (22 who received spironolactone and 11 who received amlodipine) are shown in Tables 1 and 2.

2.2. Methods

The patients were assigned randomly at a ratio of 2:1 to the spironolactone group (50 mg/d) or the amlodipine group (2.5 mg/d). These drugs were administered over a 3-month period, and blood and urine tests were performed at the beginning of this period (0 weeks) and after 12 weeks. No change was made in the administration of any drug in any patient during the 12-week (3-month) period.

In patients and healthy subjects, blood was sampled in the outpatient department from 8:30 to 9:30 AM after at least 10 hours of overnight fasting. After the blood was collected, it was immediately divided into test tubes for specific measurements, and then rapidly centrifuged at 1 500 rpm for 5 minutes to separate the serum and plasma from the clot-containing blood cells. All samples except those for glucose and glycated hemoglobin A_{1c} (HbA_{1c}) measurements were stored frozen at −70°C until analysis. At the time of blood sampling, the patients were weighed in their underwear.

2.2.1. Serum adiponectin assay

Serum adiponectin concentrations were measured in serum samples by using an enzyme-linked immunosorbent assay (ELISA) kit (Otsuka Pharmaceuticals, Tokyo, Japan). The intra- and interassay coefficients of variation (CVs) were 4.06% and 4.69%, respectively.

2.2.2. Serum visfatin assay

Visfatin was assayed in serum samples, but not in plasma, by using a visfatin C-terminal (human) EIA kit (Phoenix Pharmaceuticals, CA). The intra- and interassay CVs of this kit are less than 5% and 14%, respectively.

2.2.3. Measurement of plasma PAI-1

The plasma concentration of PAI-1 was measured in plasma in a test tube containing sodium citrate, using an ELISA (Biopool Imulyse PAI-1; Biopool, Umea, Sweden) that detects both active and latent PAI-1, as well as PAI-1 bound to tissue plasminogen activator (t-PA). The intra- and interassay CVs for the active and latent forms were 2.26% to 3.77% and 3.57% to 4.76%, respectively.

2.2.4. Serum TNF-α assay

The serum TNF-α concentration was obtained in serum samples by using an ELISA in a human TNF-α kit (JIMRO, Gumma, Japan). The intra- and interassay CVs were both less than 8.0%. In the spironolactone group, only 6 patients had a TNF-α level exceeding the lower detection limit of this assay (1.0 pg/mL) in the baseline measurement. Therefore, changes after treatment were examined only in these 6 patients.

2.2.5. Serum hsCRP and sCD40L assay

Serum hsCRP was measured by using a BN II N High-Sensitivity CRP assay (Dade Behring, Marburg, Germany), with intra- and interassay CVs of 1.72% and 2.80%, respectively, and serum sCD40L was measured by using a human sCD40L kit (Bender MedSystems, Vienna, Austria), with intra- and interassay CVs of 4.0% and 6.8%, respectively.

Table 2
Clinical characteristics and laboratory data of diabetic patients treated with spironolactone, and diabetic patients treated with amlodipine

	Spironolactone (50 mg/d)	Amlodipine (2.5 mg/d)	*P*
No. (male/female)	22 (8/14)	11 (5/6)	–
Age (y)	60.1 ± 8.0	56.5 ± 13.4	.4151
Duration (y)	12.9 ± 6.5	10.8 ± 13.5	.6279
BMI (kg/m^2)	24.6 ± 4.2	26.3 ± 5.6	.3411
FPG (mmol/L)	7.81 ± 2.55	8.88 ± 3.25	.3089
HbA_{1c} (%)	7.6 ± 1.4	8.0 ± 1.9	.5102
SBP (mm Hg)	135.5 ± 8.2	137.3 ± 9.2	.5889
DBP (mm Hg)	71.9 ± 5.7	73.4 ± 8.9	.5733
UAE (mg/g Cr)	539 (168.3, 1164.8)	295 (110, 1047)	.1747
Adiponectin (μg/mL)	14.3 ± 9.1	12.1 ± 2.3	.3061
PAI-1 (ng/mL)	22.6 ± 13.4	32.4 ± 22.3	.2038
Retinopathy (n) (NDR, SDR, PDR)	(7/4/11)	(7/1/3)	–
Statins (n)	4	5	–
Therapy (n)			
Diet	1	2	–
SU1/SU2/SU3/SU4/SU5	3/1/4/0/3	1/0/0/2/3	–
Voglibose alone	0	1	–
Insulin	10	4	–

All data except for UAE are expressed as mean ± SD, but not SE. Comparisons in variables except for UAE between the 2 groups were made by use of an unpaired *t* test. UAE is expressed as median with interquartile range (25th and 75th percentiles). Comparison in UAE between the 2 groups was made by use of a Wilcoxon rank sum test.

Table 3
Changes in adipocytokines at base line and 3 months after spironolactone therapy in patients with diabetes complicated by diabetic nephropathy

	Baseline	3 Months	*P*
Adiponectin (μg/mL)	14.3 ± 9.1	13.7 ± 7.5	.4214
HbA_{1c} (%)			
<7.5 (n = 10)	15.5 ± 11.8	13.4 ± 8.4	.1612
≥7.5 (n = 12)	13.3 ± 6.6	13.9 ± 7.1	.3264
<8.0 (n = 13)	15.9 ± 10.5	13.9 ± 7.8	.0763
≥8.0 (n = 9)	11.8 ± 6.4	13.3 ± 7.4	.0344*
<8.5 (n = 15)	14.4 ± 10.6	12.6 ± 8.1	.0685
≥8.5 (n = 7)	14.0 ± 5.4	16.0 ± 6.0	.0162*
Visfatin (ng/mL)	2.34 ± 1.18	2.52 ± 0.89	.5108
HbA_{1c} (%)			
<7.5 (n = 9)	2.83 ± 0.93	2.41 ± 0.93	.3235
≥7.5 (n = 10)	1.89 ± 1.24	2.62 ± 0.90	.0359*
<8.0 (n = 12)	2.89 ± 0.94	2.68 ± 0.98	.5451
≥8.0 (n = 7)	1.39 ± 0.92	2.26 ± 0.73	.0397*
<8.5 (n = 14)	2.59 ± 1.15	2.60 ± 0.99	.9842
≥8.5 (n = 5)	1.62 ± 1.01	2.30 ± 0.57	.0600
PAI-1 (ng/mL)	22.6 ± 13.4	19.2 ± 11.3	.0323*
HbA_{1c} (%)			
<8.0% (n = 13)	20.8 ± 9.8	19.1 ± 9.1	.3172
≥8.0% (n = 9)	25.2 ± 17.7	19.6 ± 14.4	.0565
TNF-α (pg/mL) (n = 6)	2.2 ± 1.3	4.0 ± 3.4	.1589

All data are expressed as mean ± SD, but not mean ± SE. The 2 time points for an individual were compared by use of a paired *t* test. n indicates the number of patients; 22 patients were studied, but with regard to visfatin, 19 were studied; 3 patients had been excluded because of the remarkable variation before and after therapy. TNF-α exceeded the lower value in only 6 patients at baseline. In adiponectin and visfatin, the differences were investigated also in subgroups divided by the value of HbA_{1c} (7.5%, 8.0%, and 8.5%, respectively). In PAI-1, the differences were investigated also in subgroups divided by the value of HbA_{1c} (8.0%).

* $P < .05$, statistically significant.

2.2.6. Measurement of BNP

BNP was measured in plasma stored frozen in a test tube containing disodium EDTA with an MI02 Shionogi kit (Shionogi, Osaka, Japan). The intra- and interassay CVs of this kit were both less than 15%.

2.2.7. Measurement of plasma glucose, HbA1c, and serum lipid concentrations

Fasting plasma glucose (FPG) was evaluated immediately after serum preparation, using an automated glucose oxidase method (Glucose Auto Stat GA1160, Arkray, Kyoto, Japan). The HbA_{1c} level was measured immediately after blood collection in a test tube containing dipotassium EDTA, using high-performance liquid chromatography (Hi-auto A_{1C}, HA8150, Arkray). Only HbA_{1c} is detected with this method, and the reference range is 4.3% to 5.8%. Serum total cholesterol and triglyceride concentrations were measured enzymatically.

2.2.8. Diabetic retinopathy

Diabetic retinopathy was evaluated by each patient's ophthalmologist, according to Davis' criteria [25].

2.2.9. Ethical considerations

All subjects gave their informed consent to inclusion in the study, which was performed according to the guidelines proposed in the Declaration of Helsinki. The Dokkyo University School of Medicine Ethics Committee (Koshigaya, Japan) approved the study.

Table 4
Changes in various variables at baseline and 3 months after spironolactone therapy in patients with diabetes complicated by diabetic nephropathy

	Baseline	3 Months	*P*
hsCRP (mg/L)	0.538 (0.215, 1.963)	0.437 (0.263, 0.649)	.5503
sCD40L (pg/dL)	1.2 ± 0.9	1.3 ± 0.6	.9021
BNP (μg/dL)	39.7 (12.75, 92.25)	16.25 (9.476, 53.68)	.0117*
IRI (pmol/L) (n = 12)	67.2 ± 43.2	69.6 ± 48.0	.6637
HOMA-R (n =12)	4.2 ± 2.8	4.6 ± 3.3	.4715
FPG (mmol/L)	7.81 ± 2.54	8.60 ± 2.37	.1595
HbA_{1c} (%)	7.6 ± 1.4	8.2 ± 1.5	.0025*
<8.0% (n = 13)	6.6 ± 0.8	7.5 ± 1.2	.0011*
≥8.0% (n = 9)	9.1 ± 0.6	9.3 ± 1.1	.4136
TC (mmol/L)	5.51 ± 0.94	5.59 ± 0.80	.6807
TG (mmol/L)	1.46 ± 0.67	1.36 ± 0.05	.4202
Creatinine (imol/L)	61.88 ± 8.84	79.56 ± 17.68	<.0001*
Potassium (mmol/L)	4.1 ± 0.4	4.5 ± 0.5	.0030*
UAE (mg/g Cr)	539 (168.25, 1164.75)	370 (130.25, 598.25)	.0142*
PRA (ng/L)	0.25 ± 0.27	0.54 ± 0.46	.0038*
Aldosterone (pmol/L)	281.0 ± 138.9	350.1 ± 158.9	.0157*
SBP (mm Hg)	135.8 ± 8.4	129.8 ± 8.6	.0006*
DBP (mm Hg)	72.3 ± 5.4	70.2 ± 4.4	.1207
BMI (kg/m^2)	24.8 ± 4.2	24.8 ± 4.2	.7832

All data except for hsCRP, BNP, UAE are expressed as mean ± SD, but not SE. Except for hsCRP, BNP, and UAE, the 2 time points for an individual were compared by use of a paired *t* test. HsCRP, BNP, and UAE are expressed as median with interquartile range. For hsCRP, BNP, and UAE, a Wilcoxon signed rank test as a nonparametric test was used.

n indicates the number of patients; IRI, immunoreactive insulin; HOMA-R, homeostasis model assessment ratio; TC, total cholesterol; TG, triglyceride; SBP, systolic blood pressure; DBP, diastolic blood pressure.

* $P < .05$, statistically significant.

2.2.10. Statistical methods

All data except those for hsCRP, BNP, and UAE are presented as means ± SD, but not SE, and data at the 2 time points (0 and 12 weeks) for each individual were compared using a paired *t* test. The hsCRP, BNP, and UAE data showed a skewed distribution, and are therefore expressed as medians and interquartile ranges (25th and 75th percentiles), with data at the 2 time points compared using a Wilcoxon signed rank test as a nonparametric test. Comparisons between 2 groups were made using an unpaired *t* test for data showing a normal distribution, after group normality had been confirmed by a χ^2 test; a Student *t* test or a Welch *t* test was chosen based on the homogeneity of variance calculated by an F test. For hsCRP, BNP, and UAE, a Wilcoxon rank sum test was used. A *P* value of less than .05 was accepted as indicating statistical significance.

3. Results

Three patients in the spironolactone group showed remarkable changes in visfatin levels from before to after therapy: 20.6 to 3.7, 33.1 to 0.8, and 21.1 to 1.7 ng/mL, respectively. All other patients in both groups had visfatin levels of less than 5.0 ng/mL before and after therapy, suggesting that the data for the 3 patients are rather exceptional, and that factors other than spironolactone treatment might have influenced the results. Therefore, we decided to exclude these 3 patients from statistical analysis of the visfatin data. Furthermore, because visfatin was measured in sera but not in plasma in the patients, we subsequently measured the plasma visfatin levels in 15 age-matched healthy subjects (mean age, 63.1 ± 4.7 years). There was no significant difference in the mean levels of visfatin in the sera of diabetic patients (in the spironolactone and amlodipine groups) and in the plasma of healthy subjects: 2.22 ± 1.05 vs 2.38 ± 0.79 ng/mL ($P = .5907$).

A significant decrease of PAI-1 and BNP levels and systolic blood pressure (SBP) was noted in the spironolactone group, whereas significant increases in HbA_{1c}, creatinine, potassium, plasma renin activity (PRA), and aldosterone levels were observed in these patients. The levels of adiponectin, visfatin, PAI-1 and HbA_{1c} were also investigated in subgroups of patients with HbA_{1c} less than 8.0% and 8.0% or greater, respectively; an HbA_{1c} value equal to or above 8.0% is defined by the guidelines of the Japan Diabetes Society as indicating poor diabetic control [26]. Furthermore, to confirm the validity of this cutoff value for adiponectin or visfatin, we also examined changes in the variables for subgroups divided using other HbA_{1c} cutoff values (7.5% and 8.5%). In the spironolactone group, a significant elevation of adiponectin was found in subgroups comprising patients with HbA_{1c} of 8.0% or greater and 8.5% or greater, and a significant elevation of visfatin was found in subgroups of patients with HbA_{1c} of 7.5% or greater and 8.0% or greater.

The effects of 3 months of spironolactone therapy are summarized in Tables 3 and 4, and changes in PAI-1 in all patients and changes in adiponectin in patients with HbA_{1c} 8.0% or greater are also shown for the spironolactone group in Fig. 1. In the amlodipine group, no significant changes in the measured variables were found after 3 months of treatment, except for SBP: 137.3 ± 9.2 to 125.0 ± 6.7 mm Hg ($P = .0019$).

Five patients receiving spironolactone therapy had potassium values exceeding 5.0 mmol/L; the maximum was 5.4 mmol/L. Furthermore, the number of patients with HbA_{1c} of 8.0% or greater increased from 9 to 13 after 3 months of spironolactone therapy: 4 patients with an initial HbA_{1c} less than 8.0% had HbA_{1c} 8.0% or higher after therapy. The degree of change in a variable before and after therapy was defined as the ratio of the value after 3 months to the value at baseline. Using this approach, we found no significant correlation between the PAI-1 and the SBP or body mass index (BMI) ratios ($R = -0.1318$, $P = .5588$; $R = 0.0651$, $P = .7736$, respectively) or between the HbA_{1c} and potassium ratios ($R = 0.1861$, $P = .4069$) in the spironolactone group. Finally, no correlation was obtained between PAI-1 and BMI at baseline in all 33 diabetic patients ($R = 0.1346$, $P = .4553$).

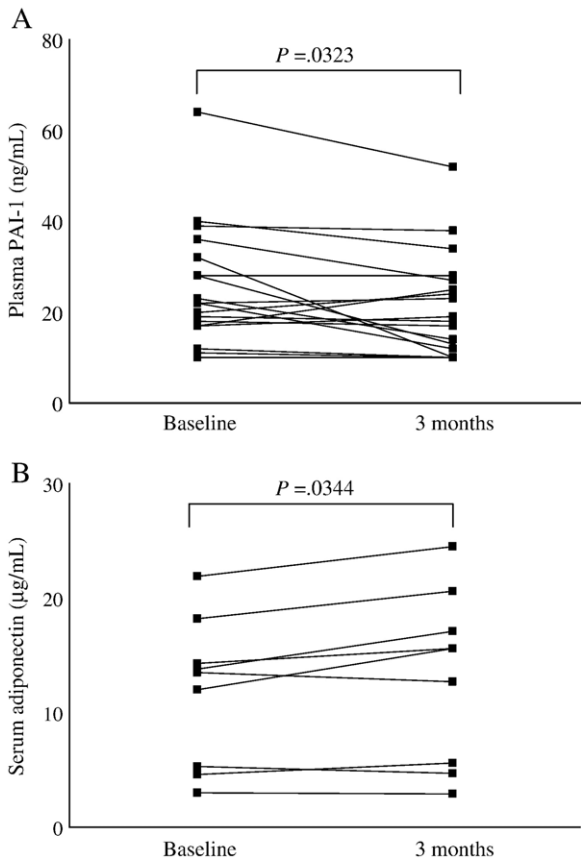

Fig. 1. A, Change in plasma PAI-1 by 3-month spironolactone therapy in the entire group of 22 diabetic patients. B, Change in serum adiponectin by 3-month spironolactone therapy in the 9 diabetic patients with poor diabetic control ($HbA_{1c} > 8.0\%$).

4. Discussion

To our knowledge, the current study is the first investigation of the effect of spironolactone, an aldosterone blocker, on adiponectin and visfatin (a recently identified adipocytokine that mimics the action of insulin [9]) in type 2 diabetes mellitus patients with diabetic nephropathy. Unlike TNF-α and PAI-1, adiponectin is expressed specifically in adipose tissue, and therefore our results may more purely reflect the effect of spironolactone in adipose tissue.

Impairment effects of A-II in various organs is partially mediated by aldosterone [16,17], and it is also reported that the MR is expressed in adipose tissue [18], in addition to the A-II type 1 receptor; therefore, we anticipated that spironolactone would increase the serum adiponectin concentration similarly to ACE-I or ARB treatment [13,14]. Contrary to our expectations, spironolactone did not influence the serum adiponectin concentration in any patients. It is difficult to explain this result, but it is of note that our patients had relatively high adiponectin concentrations before treatment, probably because of decreased clearance due to diabetic nephropathy [27]. No significant difference in adiponectin levels was observed between patients with diabetes and control subjects, although it is known that patients with diabetes generally have decreased adiponectin [28]. Therefore, the relatively elevated baseline adiponectin concentration in our patients might at least partially explain our negative findings. Furthermore, possible elevation of A-II by spironolactone therapy may have weakened its potential effect on adipocytes because spironolactone was used as a monotherapy, rather than as a combination therapy with ACE-I or ARB. It is of interest that valsartan, an ARB, has recently been shown to increase the serum adiponectin concentration only in diabetic patients (mean HbA_{1c}, 9.8% ± 2.8%) and not in nondiabetic patients [15]. In the current study, spironolactone significantly increased the adiponectin concentration in patients with poor diabetic control (HbA_{1c} ≥8.0%, as defined by the Japan Diabetes Society), although the elevation was relatively small; a similar result was found with the cutoff point changed to a higher value (HbA_{1c} ≥8.5%). Thus, our data indicate that spironolactone may influence the adiponectin concentration in a poorly controlled glycemic state through a mechanism similar to that for valsartan, although the exact mechanism is unknown. We note that these are only tentative findings, given the small sample size, and additional studies are required to confirm the validity of our results.

Regarding visfatin, although we found no significant change with either spironolactone or amlodipine therapy in all the diabetic patients, spironolactone significantly increased visfatin in patients with poor diabetic control (HbA_{1c} ≥8.0%). Similar findings occurred for cutoff points of 7.5% or 8.5%, although the increase did not reach statistical significance in patients with HbA_{1c} of 8.5% or greater. Because the physiologic role of visfatin is not completely clear, interpretation of these data is difficult. Furthermore, we should note that we measured the serum visfatin level, rather than the plasma level measured previously [9], and this and the small sample size suggest that a more detailed study is required.

We next explored the effect of spironolactone on PAI-1, the elevation of which causes impaired fibrinolysis. Spironolactone have been reported to inhibit production of PAI-1 in kidney in a rat model [29], and it has also been shown that spironolactone decreases plasma PAI-1 concentrations in patients with hypertension [30]. To our knowledge, however, the effect of spironolactone on PAI-1 in patients with diabetic nephropathy has not been investigated. We were unable to detect a significant difference between PAI-1 at baseline in diabetic patients and healthy control subjects; however, these negative findings may be due to the sample size, because it has been established that diabetic patients have elevated PAI-1 [31]. Despite the lack of a significant elevation of PAI-1 in diabetic patients, spironolactone therapy caused a significant decrease in PAI-1, especially in patients with poor diabetic control (HbA_{1c} ≥8.0%); this finding was similar to that for adiponectin or visfatin after spironolactone therapy. Although the plasma PAI-1 concentration is associated with blood pressure [30], the changes in PAI-1 and SBP were not correlated in the current study; in addition, amlodipine did not influence the PAI-1 concentration, despite causing a significant reduction in SBP. Therefore, the effect of spironolactone on PAI-1 probably occurs independently of blood pressure. It is also known that PAI-1 is associated with BMI [32]; however, the patients in the current study who had a slightly elevated BMI did not show such a correlation, and the changes in PAI-1 and BMI caused by spironolactone therapy were not correlated. Therefore, we speculate that neither BMI nor blood pressure had an influence on the change in the PAI-1 level.

We also measured the levels of TNF-α, another representative adipocytokine, in response to spironolactone therapy. Most patients had initial TNF-α levels that were less than the detection limit of the assay, but in those patients in whom TNF-α could be measured spironolactone had no effect on the TNF-α level. This suggests a weak effect of spironolactone on this cytokine, although the very small sample size makes these findings tentative. On the other hand, our data showed that spironolactone, unlike amlodipine, significantly decreased the serum BNP concentration. Spironolactone has been shown to attenuate the BNP concentration in patients with moderate CHF [33,34]. Although none of our patients had apparent clinical CHF symptoms, we did not evaluate cardiac function by ultrasonography; therefore, this is a limitation of the study, and careful interpretation of our data is required. We also explored the effect of spironolactone on hsCRP, an inflammatory marker, and on sCD40L, a newly identified predictor of cardiovascular events. Contrary to our expectations, spironolactone had no effect on these markers; therefore, we speculate that

the protective effect of spironolactone on cardiovascular events, as shown in RALES [35], is not associated with these markers.

A significant increase in creatinine level was observed only in the spironolactone group. This result may be due to a decrease in free water due to the diuretic action of spironolactone. However, this does not necessarily indicate a progression of renal impairment caused by this agent, and spironolactone may actually be rather useful for inhibiting progression of renal impairment because we also found a spironolactone-induced decrease in UAE, as shown previously [19,20]. Confirmation of these findings will require more long-term observation. Elevations of PRA and aldosterone levels were obtained with spironolactone, as expected; we speculate that this is a reactive elevation resulting from blocking of the MR by spironolactone. Furthermore, spironolactone therapy produced a significant increase in potassium, which is a reasonable result, but no patients showed a potassium concentration of more than 5.5 mmol/L, suggesting the safety of spironolactone in patients with diabetic nephropathy. However, these data are based on a limited number of patients and a short observation period, and therefore treatment of diabetic nephropathy with spironolactone should be performed only with strict monitoring of potassium.

Finally, we noticed a significant elevation of HbA_{1c} level and an increase in the number of poorly controlled patients after spironolactone therapy. It is interesting that deterioration of HbA_{1c} after spironolactone therapy was predominant in patients with HbA_{1c} less than 8.0%, and that no correlation was obtained between the HbA_{1c} ratio and the potassium concentration ratio before or after spironolactone therapy. The elevation of HbA_{1c} level in diabetic patients with diabetic nephropathy as a result of spironolactone therapy was also found in another recent report, in which spironolactone was used with an ACE-I or ARB [19]. Therefore, it is important to emphasize that spironolactone worsened diabetic control and increased the number of poorly controlled subjects, although the levels of supposedly beneficial adipocytokines were also increased.

In conclusion, we investigated the effect of spironolactone on adipocytokines in patients with type 2 diabetic nephropathy, in whom impaired endothelial function is likely to be present. We found that spironolactone therapy significantly increased serum adiponectin and visfatin in patients with poor diabetic control (HbA_{1c} >8.0%), and significantly decreased PAI-1 in all patients. Furthermore, spironolactone also significantly reduced BNP. However, our results are based on a limited period of observation, and a longer term study of the effects of this therapy is required.

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