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Effect of adiponectin on carotid arterial stiffness in type 2 diabetic patients treated with pioglitazone and metformin

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Abstract

Adiponectin, an adipocyte-specific plasma protein, has been reported to exhibit protective effects against atherosclerosis as well as an insulin-sensitizing effect. This study was designed to investigate the effect of adiponectin on carotid arterial stiffness in type 2 diabetic patients treated with pioglitazone and metformin. Twenty type 2 diabetic patients were enrolled and divided into 2 groups, a pioglitazone-treated group (n = 10) and a metformin-treated group (n = 10). Before and after intervention, plasma adiponectin levels were measured by enzyme-linked immunosorbent assay and carotid arterial stiffness was evaluated by the stiffness parameter β, measured by ultrasound equipped with a phase-locked echo-tracking system. In the pioglitazone group, plasma adiponectin level significantly increased and stiffness parameter β significantly decreased, whereas in the metformin group neither of these parameters changed significantly. The changes in stiffness parameter β were significantly and inversely correlated with change in plasma adiponectin level after treatment with pioglitazone or metformin in the group of all subjects ($r = -0.472$, $P = .036$). In conclusion, the present study is the first to demonstrate that increase in adiponectin level after treatment with the insulin sensitizers pioglitazone and metformin may improve arterial stiffness in patients with type 2 diabetes mellitus.

1. Introduction

Adiponectin, an adipocyte-specific plasma protein, exhibits protective effects against atherosclerosis as well as an insulin-sensitizing effect [1,2]. Several in vivo and vitro studies have reported that adiponectin has direct anti-atherogenic effects on the arterial wall [3,4]. Recent clinical studies found that hypoadiponectinemia was associated with endothelial dysfunction [5], cardiovascular disease [6], and diabetes mellitus [7]. Furthermore, patients with hypoadiponectinemia are at increased risk of myocardial infarction and cardiovascular death [8,9].

Pioglitazone and metformin are insulin-sensitizing agents available clinically for the treatment of type 2 diabetes mellitus. Previous clinical studies have suggested that these drugs are likely to have anti-atherogenic as well as hypoglycemic effects. Pioglitazone has been reported to increase plasma adiponectin level [10], reduce intima-media thickness (IMT) [11,12], and improve pulse wave velocity (PWV) in type 2 diabetes mellitus [13]. Metformin was also reported to improve endothelial function independent of glycemic control in type 2 diabetes mellitus after 12-week treatment [14] and to reduce IMT after 2-year treatment [15].

Arterial stiffness is one aspect of atherosclerosis, and is a functional change of arteries, as opposed to arterial wall thickening, which is a morphological change [16]. We and other authors have reported increased stiffness parameter β in patients with coronary artery disease [17] and diabetes [18-21]. Furthermore, stiffness parameter β was found to be more closely associated with peripheral circulatory parameters [19,20] than IMT. These findings suggest that the clinical implications of changes in arterial stiffness parameters are not necessarily the same as those of changes in morphological parameters of atherosclerosis such as IMT. The Atherosclerosis Risk in Communities Study first

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Table 1
Clinical characteristics at baseline and after 6-month treatment in the pioglitazone and metformin groups

	Pioglitazone		Metformin	
	Baseline	After treatment	Baseline	After treatment
n (male/female)	10 (6/4)	–	10 (5/5)	–
Age	59 ± 12	–	62 ± 5	–
Duration of diabetes	8 ± 8	–	8 ± 8	–
BMI	27.1 ± 4.3	28.3 ± 4.6***	28.5 ± 4.8	28.8 ± 4.9
SBP	130 ± 19	124 ± 19	138 ± 27	139 ± 24
DBP	79 ± 11	73 ± 13	78 ± 114	83 ± 7
FPG	175 ± 47	144 ± 41	216 ± 67	170 ± 48**
HbA_{1c}	8.3 ± 1.1	6.9 ± 0.5**	9.5 ± 2.1	8.0 ± 1.8**
IRI	10.5 ± 7.3	9.1 ± 10.3	11.0 ± 3.3	9.0 ±3.8*
HOMA-IR	5.0 ± 4.8	4.1 ± 7.0	6.0 ± 3.0	4.1 ± 2.7**
TC	205 ± 32	211 ± 47	214 ± 22	201 ± 14
TG	277 ± 242	257 ± 243	203 ± 85	169 ± 86
HDL-C	49 ± 11	48 ± 13	48 ± 11	57 ± 12**
Hs-CRP	1555 ± 1542	1616 ± 1569	1795 ± 1695	2487 ± 2681

Values are means ± SD or the number of subjects. SBP and DBP indicate systolic and diastolic blood pressures; TC, total cholesterol level; TG, triglyceride level; HDL, high-density lipoprotein cholesterol level; Hs-CRP, high-sensitive C-reactive protein.

* $P < .05$ vs baseline data.

** $P < .01$ vs baseline data.

*** $P < .001$ vs baseline data.

demonstrated that the arterial stiffness parameter β of the common carotid artery increased with fasting hyperinsulinemia, suggesting a close association between this arterial property and insulin resistance [22]. We subsequently found that the stiffness parameter β of both the carotid and femoral arteries were more strongly associated with insulin resistance in type 2 diabetes mellitus than IMT, in a euglycemic hyperinsulinemic glucose clamp study [18,23]. Furthermore, short-term aerobic exercise also improved stiffness parameter β of both arteries with improvement of insulin resistance [24]. We recently found an inverse association of stiffness parameter β of the carotid artery with plasma adiponectin level in a cross-sectional study [25]. These previous studies suggest the possibility that drugs modifying insulin resistance and/or adiponectin may improve arterial stiffness. However, no reports are available concerning the direct effects of the insulin sensitizers pioglitazone and metformin on stiffness parameters. The aim of the present prospective study was to investigate whether the insulin sensitizers pioglitazone and metformin improve arterial stiffness via changes in adiponectin level in type 2 diabetes mellitus.

2. Research design and methods

2.1. Subjects

Twenty type 2 diabetic subjects (11 men and 9 women) were enrolled in the present study at the Diabetes Center of Osaka City University Hospital. The mean age and duration (±SD) of diabetes were 61 ± 9 and 8 ± 8 years, respectively. The body mass index (BMI) was 27.8 ± 4.6 kg/m^2. The subjects were divided into pioglitazone- and metformin-treated groups. Ten type 2 diabetic subjects in each group took pioglitazone or metformin for 6 months. In the pioglitazone group, 5 subjects were administered 15 mg/d and 5 subjects 30 mg/d. In the metformin group, 5 subjects were administered 500 mg/d and 5 subjects 750 mg/d. Before treatment, the subjects were treated with sulfonylureas, α-glucosidase inhibitor, or medical nutritional therapy alone, with the regimens of these treatments maintained without changes in dose during this study. Before and after intervention, we evaluated plasma adiponectin level and stiffness parameter β, to measure the stiffness of the common carotid arteries, by ultrasound.

2.2. Stiffness parameter β

Ultrasonic examinations of stiffness parameter β of the common carotid arteries were performed in the supine position with slight hyperextension of the neck using an ultrasonic phase-locked echo-tracking system equipped with a high-resolution, real-time 13-MHz linear scanner (Pro-Sound SSD 6500, Aloka, Tokyo, Japan) as previously reported [18-21]. Stiffness parameter β, an index of the elastic properties of the arterial wall, was calculated using the blood pressure and diameter of the artery as follows: stiffness parameter $\beta = [\ln (Ps/Pd)] \times Dd/(Ds - Dd)$, where Ps and Pd are systolic and diastolic blood pressures and Ds and Dd are the systolic and diastolic inner diameters of the artery, respectively.

2.3. Assay of plasma adiponectin and biochemical analysis

Plasma adiponectin levels were measured using an enzyme-linked immunosorbent assay kit (Otsuka Pharmaceuticals, Tokyo, Japan) while each patient was fasting. Plasma glucose levels were measured using the glucose oxidase method, HbA_{1c} by high-pressure liquid chromatography (reference range, 4.0%-5.8%), and plasma insulin levels by immunoradiometric assay (Insulin Riabead II kit;

Dainabot, Tokyo, Japan). Serum total cholesterol, triglyceride, and high-density lipoprotein cholesterol levels were measured using enzymatic methods adapted to an autoanalyzer (Hitachi 7450; Hitachi, Tokyo, Japan). Plasma high-sensitive C-reactive protein levels were measured by nephelometric assay (Behring Nephelometer Analyzer II; Behring, Marburg, Germany). The insulin resistance index by homeostasis model assessment (HOMA-IR) was calculated from fasting plasma glucose (FPG) and insulin (IRI) levels according to a report by Matthews et al with the formula: HOMA-IR = IRI in mU/L × FPG (in mg/dL)/405 [26-28].

2.4. Statistical analysis

Values are presented as means ± SD unless otherwise indicated. Statistical analyses were performed with StatView 5.0 software (SAS Institute, Cary, NC). The paired and unpaired t tests, χ^2 tests, and simple linear regression analysis were used where appropriate. P values of less than .05 were considered to indicate statistical significance.

3. Results

Table 1 shows the clinical characteristics of the pioglitazone- and metformin-treated groups before and after treatment. There were no significant differences in baseline characteristics between the pioglitazone and metformin groups. From before to after treatment, BMI increased significantly in the pioglitazone group but did not change in the metformin group. In the pioglitazone group, HbA_{1c} significantly decreased and FPG, IRI, and HOMA-IR tended to decrease, although not to significant extents. In the metformin group, FPG, HbA_{1c}, IRI, and HOMA-IR decreased significantly. Systolic and diastolic blood pressures, total cholesterol level, and triglyceride level were changed by treatment in neither group. High-density lipoprotein cholesterol level increased significantly in the metformin group. High-sensitive C-reactive protein changed in neither group.

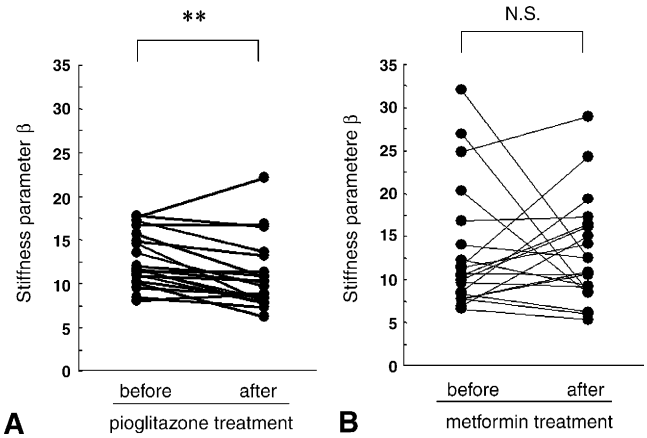

Fig. 2. Changes in stiffness parameter β in the right and left carotid arteries in the pioglitazone and metformin groups. Stiffness parameter β was significantly decreased in the pioglitazone group, but not in the metformin group. **P < .01 vs before treatment in each group.

Plasma adiponectin level increased significantly in the pioglitazone group (4.9 ± 1.8 to 10.2 ± 5.4 μg/mL, P = .0027), but not the metformin group (5.1 ± 2.7 to 6.7 ± 4.9 μg/mL, P = .061) (Fig. 1). Stiffness parameter β of the right and left common carotid arteries decreased significantly in the pioglitazone group (12.9 ± 3.1 to 11.1 ± 3.9, P = .0025), but not in the metformin group (13.7 ± 7.1 to 13.5 ± 5.9, P = .944) (Fig. 2). We also analyzed changes in plasma adiponectin level and stiffness parameter β for each different dose of pioglitazone or metformin. In subjects treated with 30 mg/d of pioglitazone, plasma adiponectin level increased (4.9 ± 2.2 to 12.0 ± 5.1 μg/mL, P = .016) and stiffness parameter β decreased (12.4 ± 2.7 to 9.8 ± 2.9, P = .007) significantly. In subjects treated with 15 mg/d of pioglitazone, the changes of neither plasma adiponectin level (4.8 ± 1.6 to 8.6 ± 5.8 μg/mL, P = .113) nor stiffness

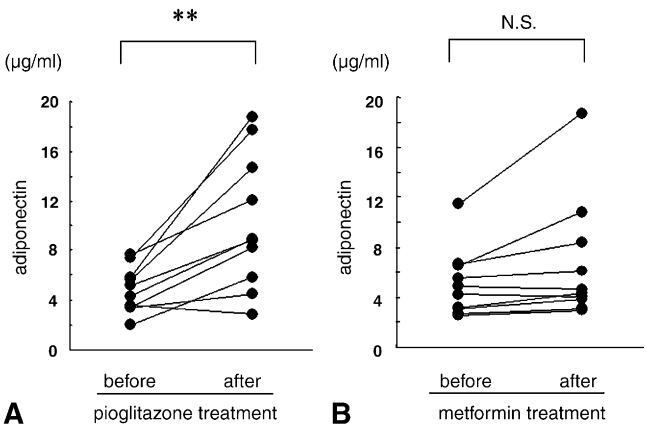

Fig. 1. Changes in plasma adiponectin level in the pioglitazone and metformin groups. Plasma adiponectin level significantly increased in the pioglitazone group, but not in the metformin group. **P < .01 vs before treatment in each group.

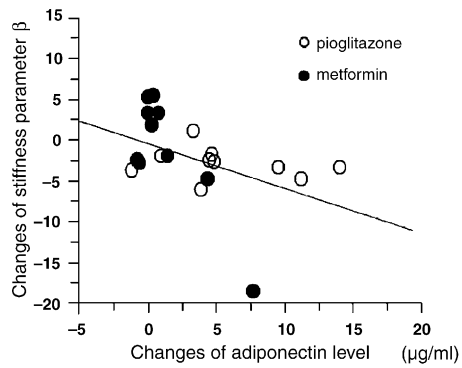

Fig. 3. Relationship between change in plasma adiponectin level and change in arterial stiffness after treatment with pioglitazone and metformin in the group of all subjects. The mean of change bilaterally in arterial stiffness parameter β as significantly and inversely correlated with change in plasma adiponectin level in the group of all subjects (r = −0.472, P = .036).

parameter β (13.5 ± 3.4 to 12.3 ± 4.6, $P = .149$) reached statistical significance. We failed to find statistically significant changes in plasma adiponectin level or stiffness parameter β in subjects treated with either 500 or 750 mg/d of metformin (500-mg group: adiponectin, 4.2 ± 1.6 to 5.2 ± 3.2 μg/mL [$P = .302$]; stiffness parameter β, 13.1 ± 6.0 to 14.3 ± 7.9 [$P = .588$]; 750-mg group: adiponectin, 5.9 ± 3.5 to 8.1 ± 6.2 μg/mL [$P = .158$]; stiffness parameter β, 14.3 ± 8.3 to 12.8 ± 3.2 [$P = .636$]).

To explore the effects of plasma adiponectin level on stiffness parameter β, we examined the relationship between change in plasma adiponectin level and the mean value of change in stiffness parameter β for the 2 carotid arteries in each subject after treatment with pioglitazone or metformin for the group of all subjects. The mean value of change in bilateral arterial stiffness parameter β was significantly and inversely correlated with change in plasma adiponectin level ($r = -0.472$, $P = .036$) (Fig. 3).

4. Discussion

The present study demonstrated that 6-month pioglitazone treatment improved arterial stiffness in type 2 diabetic patients. The changes in stiffness parameter β in the metformin group varied widely and did not reach statistical significance. In the group of all patients, improvement of stiffness parameter β was significantly associated with change in plasma adiponectin level for both pioglitazone and metformin.

Recent in vivo and in vitro studies have suggested that adiponectin plays a role in protecting against the development of atherosclerosis [3,4,29]. Clinically, hypoadiponectinemia can be used as a predictor of the incidence of coronary artery disease in subjects with coronary artery stenosis [6] and without previous history of cardiovascular disease [9]. Plasma adiponectin level was previously found to be associated with endothelium-independent vasodilatation in healthy subjects [30] and forearm blood flow in Japanese subjects without history of cardiovascular disease or diabetes [5].

The stiffness parameter β assessed by ultrasound represents the elastic properties of the arterial wall, which are affected by increase in collagen content and decrease in elastin content resulting from pathologic structural changes [31-33]. This parameter worsens in association with aging [31] and coronary artery disease [17]. We previously reported that in type 2 diabetic patients, stiffness parameter β is associated with various pathologic features of diabetes, including decreased glomerular filtration rate [34], peripheral vascular disease [19,20], and angiotensin-converting enzyme (ACE) polymorphism [35]. In the Atherosclerosis Risk in Communities Study, stiffness parameter β of the common carotid artery was first demonstrated to increase with fasting hyperinsulinemia [22], indicating a close relationship between this arterial property and insulin resistance. We subsequently demonstrated that stiffness parameter β of the carotid and femoral arteries was closely correlated with insulin resistance in a euglycemic hyperinsulinemic clamp study [18] and that aerobic exercise improved stiffness parameter β at least in part in accordance with improvement in insulin resistance [24]. Recently, in a cross-sectional study, we found an inverse association between stiffness parameter β of the carotid artery and plasma adiponectin level independent of known atherogenic factors [25]. These previous findings suggest the usefulness of intervention to decrease arterial stiffness with insulin sensitizers via the effect of adiponectin.

The present study revealed that stiffness parameter β was decreased with increase in plasma adiponectin level in patients treated with pioglitazone and metformin. These findings suggest that adiponectin may contribute to arterial stiffness in type 2 diabetes mellitus. Two possible mechanisms linking adiponectin and arterial stiffness can be considered. First, adiponectin may affect arterial stiffness via insulin resistance. In in vivo studies, administration of adiponectin improved hepatic and peripheral insulin resistance [1,2]. In human studies, adiponectin level was found to be correlated with insulin resistance [36]. We previously showed that stiffness parameter β of the carotid and femoral arteries is determined at least in part by insulin resistance [18]. Second, adiponectin may have direct anti-atherogenic effects on the arterial wall. In in vitro studies, adiponectin was found to inhibit expression of endothelial adhesion molecules in endothelial cells [4], reduce atherogenic transformation of macrophages [29], and inhibit vascular smooth muscle proliferation [3].

Several studies have demonstrated that thiazolidinediones including pioglitazone increase plasma adiponectin level [10,37-39], although a few found that metformin did not significantly affect plasma adiponectin level [38,39]. In addition, several clinical studies have found that pioglitazone improves surrogate indices of atherosclerosis, IMT, and pulse wave velocity in type 2 diabetic subjects [11,13]. Our findings are consistent with the results reported in these previous studies. In our metformin-treated subjects, no significant changes in arterial stiffness were found despite improvement of insulin resistance, although a few studies have found that metformin also improved endothelial function in type 2 diabetes mellitus [14] and reduced IMT [15]. The significant correlation between increase in adiponectin level and improvement of arterial stiffness (Fig. 3) suggests that adiponectin affects arterial stiffness more strongly than insulin resistance per se.

There are a few limitations to our study. First, the number of subjects was small. We are therefore unable to draw definite conclusions concerning the effects of metformin on arterial stiffness and plasma adiponectin level and sex differences in our findings. Second, the subjects were treated with 2 different doses of pioglitazone or metformin to avoid adverse effects of each drug. In Japanese subjects, edema of the leg may occur with pioglitazone treatment and gastrointestinal symptoms with metformin treatment

even with relatively lower doses of each drug. These limitations make it difficult to clearly determine the findings associated with different doses of each drug. A further study with a larger number of subjects will be needed to address these limitations.

In conclusion, plasma adiponectin affects carotid arterial stiffness, and agents targeting plasma adiponectin level such as thiazolidinediones may be effective in decreasing the rate of development of atherosclerosis in type 2 mellitus. Further studies are needed to clarify the clinical effects of the various modulators of adiponectin on arterial stiffness.

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