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Pioglitazone Increases Serum DPP-4 Level in Type 2 Diabetes Mellitus

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Abstract

Objective: The aim of this study is to clarify the effect of pioglitazone on serum Dipeptidyl peptidase-4 (DPP-4) level in type 2 diabetes mellitus.

Methods: Chronological changes in serum DPP-4 level were observed in 22 patients treated with pioglitazone and 15 with metformin during 12 months. Serum DPP-4 concentrations were measured by an ELISA kit.

Results: In the pioglitazone group, serum DPP-4 levels were significantly increased from baseline (774 ± 198 ng/mL) with mean changes of 53 mg/mL [6.8% increase, 95% confidence interval (CI) 2 to 104, $p<0.05$] after 3 months and of 74 mg/mL (9.4% increase, 95% CI 21 to 126, $p<0.01$) after 12 months. On the other hand, serum DPP-4 levels were not changed in the metformin group. Percent change in serum DPP-4 level was significantly and positively correlated with percent change in body mass index ($r=0.39$, $p<0.02$). The association was still significant even after adjusting for age, duration of diabetes, serum creatinine, and HbAc1 ($\beta=0.59$, $p<0.02$).

Conclusion: The present study showed that pioglitazone, but not metformin, chronologically increased serum DPP-4 levels during 12 months.

Keywords: Pioglitazone; Dipeptidyl Peptidase-4; Type 2 diabetes mellitus; Adipocyte; PPAR-γ; PPAR-γAgonist; Metformin; Body Mass Index

Abbreviations: PPAR-γ: Peroxisome proliferator-activated receptor-γ; DPP-4: Dipeptidyl peptidase-4; HbA1c: Hemoglobin A1c; BMI: Body mass index; CI: Confidence interval

Introduction

Pioglitazone is a peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist, and is widely used for treatment of patients with type 2 diabetes mellitus. Pioglitazone is known to increase insulin sensitivity by activating PPAR-γ in adipose tissue and to improve glycemic control [1]. Pioglitazone is considered to be unique in its ability to reduce a progression of atherosclerosis and to prevent an occurrence of cardiovascular events partly through increasing the action of adiponectin, which is one of the adipocytokines specifically and highly expressed in visceral and subcutaneous fat depots, by inducing the differentiation of adipocytes [2-5].

DipeptidylPeptidase-4 (DPP-4) is a ubiquitously expressed transmembrane glycoprotein that cleaves N-terminal dipeptides from a variety of substrates including incretin such as glucagon-like peptide-1 and gastric inhibitory polypeptide, which are released from the intestinal mucosa [6,7]. DPP-4 has gained considerable interest as a therapeutic target, and DPP-4 inhibitors that prolong the insulinotropic effect of incretin are now available for treatment of type 2 diabetes in clinical settings. Recently, it is reported that DPP-4 is expressed in adipocytes during their differentiation along with adiponectin expression and may impair insulin sensitivity directly in fat, skeletal, and smooth muscle cells [8]. *In vitro* experiments demonstrated that DPP-4 concentration tended to be increased by the treatment with PPAR-γ agonists [8]. These findings suggest that serum DPP-4 levels may be affected by modulating adipocyte differentiation. However, it is unknown whether or not pioglitazone affects serum levels of DPP-4 in human. Therefore, we conducted a clinical study to examine whether or not serum DPP-4 levels are affected by PPAR-γ agonist in type 2 diabetes.

Subjects and Methods

Subjects

This is an observational study with 22 patients treated with pioglitazone and 15 patients with metformin, who visited Shimane University Hospital for treatments of type 2 diabetes. The patients were enrolled if informed consent was obtained after a detailed explanation of the study purpose and methods. None of them had hepatic or renal dysfunction, and taken thiazolidinedione, biguanides, or DPP-4 inhibitors so far. Pioglitazone (15-30 mg) was orally administered once daily and metformin (250mg) was two or three times after meal (500-750 mg/day) throughout 12 months. The numbers of patients who had been taking insulin, sulfonylurea, and alpha-glucosidase inhibitors were 12, 6, and 3 in the pioglitazone group, and 9, 4, and 1 in the metformin group. All prescription medications of each patient were not changed during this study. This study was approved by the ethical review board of Shimane University Faculty of Medicine, and complied with the Helsinki Declaration.

Biochemical measurements

After an overnight fasting, blood sample were collected. Biochemical markers were measured by standard methods. Hemoglobin A1c (HbA1c) was determined by high performance liquid chromatography. The value for HbA1c (%) is estimated as an NGSP (National Glycohemoglobin Standaridization Program) equivalent value (%) calculated by the formula; HbA1c (%) = HbA1c (JDS) (Japan Diabetes Society) (%) + 0.4 % [9].

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Serum DPP-4 concentration was measured by using ELISA kits purchased from R&D Systems (Minneapolis, MN). The assays were performed in duplicates according to the manufacturer's instructions. In brief, 96 wells of a polystyrene microplate were coated with anti-DPP-4 monoclonal antibody. Fifty µl of serum samples was placed in each of the 96 wells. After 2-hour incubation at room temperature, the polyclonal antibody against DPP-4 conjugated with horseradish peroxidase was used as the detecting antibody. Contents of wells were incubated for further 2 hours with substrate solution. After the reaction was stopped, the absorbance was measured at 450 nm within 30 minutes. The coefficient of variation of measurements of DPP-4 was <10.0%.

Statistical analysis

Data were expressed as mean ± SD. Wilcoxon tests were used to evaluate the effect of pioglitazone and metformin on body weight, waist circumference, HbA1c, and DPP-4 levels as compared with the data obtained at baseline. Simple correlation and multiple regression analyses were used for the relationships between two parameters. All analyses were carried out using the statistical computer program Stat View (Abacus Concepts, Berkeley, CA). P<0.05 was considered to be significant.

Results

Baseline characteristics of patients and comparison of parameters between the pioglitazone and the metformin groups

A total of 37 patients were enrolled, with none of them withdrawing from the study. We compared various variables between the pioglitazone and the metformin groups (Table 1). No significant differences in all variables were found between them.

Chronological changes in serum DPP-4 levels, body mass index, waist circumference, and HbA1c

Chronological changes in serum DPP-4 levels, Body Mass Index (BMI), waist circumference, and HbA1c were shown in Table 2. In the pioglitazone group, BMI, and waist circumference were significantly and consecutively increased at 3 and 12 months after treatment with pioglitazone (p<0.05), while HbA_{1c} was significantly decreased at 3 and 12 months (p<0.01). Serum DPP-4 levels significantly increased from baseline with mean changes of 53 ng/mL [6.8% increase, 95% confidence interval (CI) 2 to 104, p<0.05] at 3 months and of 74 ng/mL (9.4% increase, 95%CI 21 to 126, p<0.01) at 12 months after treatment with pioglitazone. On the other hand, BMI, waist circumference, and serum DPP-4 levels were not changed after treatment with metformin, while HbA1c was significantly decreased at 3 and 12 months (p<0.05). Changes in serum DPP-4 level at 12 months were significantly greater in the pioglitazone group than in the metformin group (Figure 1).

	pioglitazone	Metformin	*p*
Number of subjects	22	15	
Sex (Male/Female)	14/8	10/15	
Age (years)	67.1 ± 10.1	66 ± 10.4	0.742
Duration of diabetes (years)	14.3 ± 9.1	15.1 ± 13.1	0.843
Body mass index (kg/m^2)	22 ± 2.3	23.2 ± 1.7	0.102
Waist circumference (cm)	84.4 ± 7.4	87.4 ± 7.2	0.238
Serum creatinine (mg/dL)	0.77 ± 0.20	0.79 ± 0.20	0.793
HbA1c (%)	7.9 ± 1.7	7.5 ± 1.0	0.332
DPP-4 (ng/mL)	774 ± 198	858 ± 199	0.211

Data are means ± SD. P values were calculated using Students *t*-tests.

HbA1c: Hemoglobin A1c; DPP-4: Dipeptidyl peptidase-4

Table 1: Baseline characteristics of the patients and comparison of parameters between the pioglitazone and the metformin groups.

pioglitazone	baseline	3 months	12 months
Body mass index (kg/m^2)	22 ± 2.3	22.5 ± 2.2**	23.4 ± 2.6***
Waist circumference (cm)	84.4 ± 7.4	85.9 ± 7.4*	87.7 ± 8.5**
HbA1c (%)	7.9 ± 1.7	7.4 ± 1.7**	7.1 ± 1.2***
DPP-4 (ng/mL)	774 ± 198	827 ± 216*	847 ± 210*
metformin			
Body mass index (kg/m^2)	23.2 ± 1.7	23.3 ± 1.7	23.5 ± 2.1
Waist circumference (cm)	87.4 ± 7.2	87.6 ± 7.3	88.2 ± 7.3
HbA1c (%)	7.5 ± 1.0	6.9 ± 0.8*	6.9 ± 0.9*
DPP-4 (ng/mL)	858 ± 199	854 ± 229	846 ± 216

Data are means ± SD. P values were calculated using Wilcoxon tests.
HbA1c: Hemoglobin A1c; DPP-4: Dipeptidyl peptidase-4.
*; p<0.05, **; p<0.01, ***; p<0.001

Table 2: Chronological changes in body weight, waist circumference, HbA1c and DPP-4.

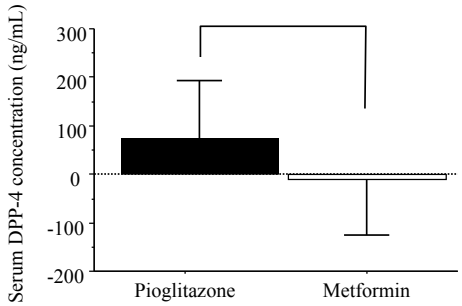

Figure 1: The difference in changes in serum DPP-4 levels between the pioglitazone and metformin groups

Changes in serum DPP-4 levels in patients with pioglitazone were significantly greater than those in them with metformin. *; p<0.05.

	%change in DPP-4	
	r	*p*
Age	-0.03	0.841
Duration of diabetes	0.15	0.367
Serum creatinine	-0.14	0.429
Body mass index	-0.29	0.085
%change in body mass index	0.39	0.017
Waist circumference	-0.21	0.213
%change in waist circumference	0.21	0.204
HbA1c	0.06	0.718
%change in HbA1c	0.2	0.247

HbA1c: Hemoglobin A1c; DPP-4: Dipeptidyl peptidase-4

Table 3: Correlation between percentage change in serum DPP-4 levels and other variables.

Correlations between percent change in serum DPP-4 concentration versus baseline and changes in values of each parameter

We performed correlation analysis between percent (%) change in serum DPP-4 concentration versus baseline and % change in values of each parameter at 12 months in order to investigate which parameters could be associated with serum DPP-4 levels (Table 3). % change in

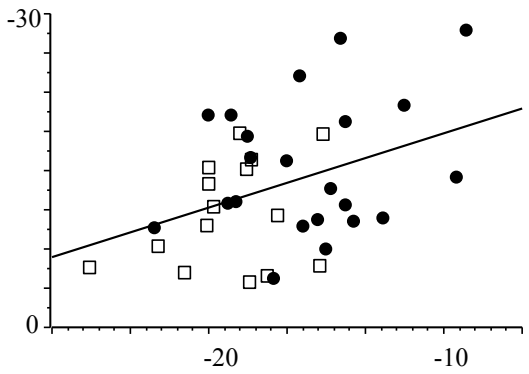

Figure 2: The correlation between %change in serum DPP-4 level and %change in body mass index

%change in serum DPP-4 level was significantly and positively correlated with %change in body mass index (r=0.39, p=0.017). Black circles indicate pioglitazone, and white squares indicate metformin.

DPP-4 was significantly and positively correlated with % change in BMI (Figure 2), but not other parameters. Then, multiple regression analysis adjusted for age, duration of diabetes, serum creatinine, and HbA1c showed that the positive association between % change in DPP-4 and % change in BMI was still significant (β=0.59, p=0.013).

Discussion

DPP-4 is considered as a negative regulator of glucose tolerance by inactivating incretin action. In addition, blood levels of DPP-4 were elevated in poorly controlled diabetes [10]. To our knowledge, there are no clinical studies investigating the effect of PPAR-γ agonists on serum DPP-4 levels. In this study, we found for the first time that pioglitazone increased serum DPP-4 levels in type 2 diabetes.

A recent study showed that DPP-4 is expressed in adipocytes and induces insulin resistance directly, and that serum DPP-4 levels were associated with visceral fat accumulation and metabolic syndrome [8]. These data suggest that DPP-4 is one of the adipocytokines and that serum DPP-4 levels may be affected by adipogenesis. Since PPAR-γ is a master regulator of adipogenesis and its activation induces mesenchymal stem cells into adipocytes, PPAR-γ agonists might affect DPP-4 expression and secretion in adipocytes. In this study, serum DPP-4 levels were slightly but significantly increased after treatment with pioglitazone, while they were not changed in patients treated with metformin. Moreover, changes in serum DPP-4 levels were associated with changes in BMI. These findings suggest that increased serum DPP-4 by pioglitazone might be derived from adipose tissue.

The relationships of serum DPP-4 increased by pioglitazone with glucose metabolism and diabetic complications is unknown. Previous studies indicated that treatment with pioglitazone decreased the incidence of cardiovascular disease [2]. The favorable effects of pioglitazone on cardiovascular disease might be its pleiotropic effect. On the other hand, pioglitazone is reported to increase the risks of heart failure and osteoporotic fracture [11,12]. Several studies showed that inhibitions of DPP-4 improved heart failure after cardiovascular infarction as well as osteoporosis fractures [13,14]. Although the mechanism of the increased risks of heart failure and osteoporotic fracture by pioglitazone is still discussed, these findings suggest that increased DPP-4 levels by pioglitazone might be involved in the pioglitazone-related adverse events. A previous animal study showed that DPP-4 inhibitors suppressed pioglitazone-induced gain of body weight, suggesting that combined therapy of pioglitazone with DPP-4 inhibitors might be useful to lead the beneficial effects of pioglitazone without adverse effects [15].

In conclusion, this is the first report showing that pioglitazone treatment increased serum levels of DPP-4 in patients with type 2 diabetes. However, this is a small observational study to investigate the effect of pioglitazone on serum DPP-4 levels. We thus need further large scale clinical trials to confirm our findings.

Author Contributions

Authors' roles: Conceived and designed the study: Ippei Kanazawa. Corrected and analyzed the data: Sayuri Tanaka, Masakazu Notsu. Contributed equipment/materials: Toshitsugu Sugimoto. Wrote the paper: Ippei Kanazawa. Approving final version: All authors

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