Investigation of Pharmacological Responses to an Anti-Diabetic Drug Pioglitazone in Female Spontaneously Diabetic Torii (SDT) Fatty Rats, A New Obese Type 2 Diabetic Rat

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Abstract

Introduction: Rigorous glycemic control is essential to prevent the development of diabetes and onset of diabetic complications. Peroxisome proliferator-activated receptor (PPAR) γ activator pioglitazone is an anti-diabetic drug that exhibits glucose-lowering effects by enhancing insulin sensitivity in peripheral tissues. However, increases in body weight are a concerning finding associated with treatment with pioglitazone. In this study, the pharmacological effects of pioglitazone were investigated in female Spontaneously Diabetic Torii (SDT) fatty rats, a new obese type 2 diabetic rat, to verify utility of this model.

Methods: Pioglitazone was administered to SDT fatty rats from 7 to 13 weeks of age, and changes in body weight and blood chemical parameters, fat distribution using Computed tomography (CT) analysis, and histopathological analyses of the pancreas, kidney, and liver, were evaluated.

Results: Pioglitazone treatment significantly decreased blood glucose, hemoglobin A1c, and triglyceride levels. However, increases in body weight were observed. In CT analysis, both visceral and subcutaneous fat weights also increased. In SDT fatty rats, histopathological changes, such as irregular boundaries and vacuolation of pancreatic islets, renal tubular vacuolation and extension, and hepatocellular fatty changes and hypertrophy, were observed. Pioglitazone treatment improved histopathological abnormalities.

Conclusion: In female SDT fatty rats, the sub chronic administration of pioglitazone improved hyperglycemia, hypertriglyceridemia, and histopathological lesions in the pancreas, kidney, and liver. However, treatment induced increases in the body weight. Female SDT fatty rats are useful for the development of new anti-diabetic drugs which show potential to enhance insulin sensitivity.

Keywords
SDT fatty rat, Pioglitazone, Blood chemical parameters, Fat distribution, Histopathological changes

Abbreviations
CT: Computed Tomography; DPP: Dipeptidyl Peptidase; HbA1c: Hemoglobin A1c; HE: Hematoxylin and Eosin; NASH: Nonalcoholic Steatohepatitis; PPAR: Peroxisome Proliferator-activated Receptor; SDT: Spontaneously Diabetic Torii; TC: Total Cholesterol; TG: Triglyceride; VEGF: Vascular Endothelial Growth Factor; V/S: Visceral/Subcutaneous; ZDF: Zucker Diabetic Fatty

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the onset of these complications. Epidemiological mega-studies, such as the Diabetes Control and Complications trial and the UK Prospective Diabetes Study, have demonstrated significant benefit with long-term intensive control of blood glucose levels [8,9]. Since there are various critical factors causing diabetic patients, including genetic and environmental factors, several anti-diabetic drugs with differing mechanisms have been developed [10]. Peroxisome proliferator-activated receptor (PPAR) γ activator pioglitazone is an anti-diabetic drug that exhibits glucose-lowering effects by enhancing insulin sensitivity in peripheral tissues, thereby contributing to diabetes management. Furthermore, a prior meta-analysis showed that pioglitazone reduced the risk of myocardial infarction, stroke and death in patients with type 2 diabetes [11].

Diabetic animal models are essential for obtaining a better understanding of diabetes mellitus and developing novel anti-diabetic drugs. The Spontaneously Diabetic Torii (SDT) fatty rat was established by introducing the fa allele of the Zucker fatty rat into the SDT rat genome [12]. The male SDT fatty rat presents with obesity, hyperglycemia and dyslipidemia at a young age compared with the male SDT rat. Furthermore, with the early incidence of diabetes mellitus, diabetes-associated complications in the SDT fatty rat are seen at younger age compared with the SDT rat [13]. The female SDT fatty rat also develops diabetes and its complications at a young age and has the potential to become an important animal model for obese type 2 diabetes, especially for women, for which few models currently exist [14]. In fact, the SDT fatty rat is considered to be a suitable model to help understand the properties of type 2 diabetes with obesity. Furthermore, in addition to pathophysiological analyses of diabetes and its complications in the diabetic model, investigating the pharmacological effects of anti-diabetic drugs to elucidate the properties of these animals as a diabetic animal model is important. In this study, pioglitazone was repeatedly administered to female SDT fatty rats, and the effects of pioglitazone were investigated.

**Material and Methods**

Female SDT fatty rats were purchased from CLEA Japan Inc. (Tokyo, Japan). Rats were housed in suspended bracket cages and given a standard laboratory diet (CRF-1, Oriental Yeast Co., Ltd. Tokyo, Japan) and water ad libitum in a controlled room for temperature, humidity and lightning. Pioglitazone (1, 10 mg/kg) was administered to SDT fatty rats from 7 to 13 weeks of age (n = 5). The drug, suspended in 0.5% methyl cellulose (MC) solution, was administered orally by means of a stomach tube at a volume of 5 mL/kg.

Body weights, and non-fasting serum biochemical parameters, such as glucose, insulin, triglyceride (TG), and total cholesterol (TC) levels, were evaluated every two weeks. Hemoglobin A1c (HbA1c) levels were also determined at 13 weeks of age. Glucose, TG, TC, and HbA1c levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and an automatic analyzer (Hitachi, Tokyo, Japan). Serum insulin levels were measured using rat-insulin enzyme-linked immunosorbent assay (ELISA) kits (Morinaga Institute of Biological Science, Yokohama, Japan).

Computed tomography (CT) analyses were taken at 13 weeks of age, and visceral fat, subcutaneous fat and lean body mass weights were determined. Weights were measured using a laboratory X-ray CT device (LA Theta, ALOKA Co., LTD., Osaka, Japan). Rats were anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital (Tokyo Chemical Industry, Tokyo, Japan), and approximately 20 CT photographs in a rat were taken at 5 mm intervals between the diaphragm and lumbar vertebrae of the rats. Total fat weight and visceral/subcutaneous (V/S) ratios were calculated based on visceral and subcutaneous fat weights.

Necropsies were performed at 13 weeks of age. After measuring the weights of kidneys and livers, the organs including the pancreas were fixed in 10% neutral buffered formalin. After resection, the tissues were paraffin-embedded using standard techniques and thin-sectioned (3 to 5 µm). The sections were stained with hematoxylin and eosin (HE).

Results of biological parameters were expressed as means ± standard deviation. A statistical analysis of differences between mean values in the control group and pioglitazone-treatment groups was performed using a One-way analysis of variance (ANOVA) followed by Dunnett’s two-tailed test.

**Results**

Changes in body weight and biochemical parameters are shown in Figure 1. Body weight in the pioglitazone 10 mg/kg group significantly increased after 11 weeks of age (Figure 1A). Serum glucose and TG levels in the pioglitazone 10 mg/kg group significantly increased after 11 weeks of age (Figure 1A). Serum glucose and TG levels in the pioglitazone 10 mg/kg group significantly increased after 11 weeks of age (Figure 1A).
pared with those in the control group. The fat rate also increased; however, the V/S ratio in the pioglitazone 1 and 10 mg/kg groups decreased. Lean body mass in the pioglitazone 1 and 10 mg/kg groups decreased significantly compared with that in the control group.

Histopathological changes in the pancreas, kidney, and liver are shown in Figure 2, Figure 3 and Figure 4. Since the biochemical parameters in the pioglitazone 1 mg/kg group were not improved, the histopathological analysis was not performed. Histopathological abnormalities, such as irregular boundaries, atrophy, and vacuolation of islets, were observed in the pancreas of animals in the control group. These changes in the pancreas were inhibited with pioglitazone treatment at a dose of

Table 1: Effects of pioglitazone on fat tissue weights in female SDT fatty rats at 13 weeks of age.

|                          | Visceral fat (g) | Subcutaneous fat (g) | Total fat (g) | V/S ratio | Lean body mass (g) | Fat rate (%) |
|--------------------------|------------------|----------------------|---------------|-----------|--------------------|--------------|
| Control group            | 62.7 ± 5.8       | 56.7 ± 17.8          | 119.3 ± 23.3  | 1.17 ± 0.25 | 151.1 ± 7.2        | 45.3 ± 4.3   |
| Pioglitazone 1 mg/kg group | 61.2 ± 9.7       | 70.3 ± 20.3          | 131.5 ± 29.5  | 0.90 ± 0.13* | 137.9 ± 6.8*       | 48.3 ± 4.9   |
| Pioglitazone 10 mg/kg group | 84.8 ± 4.4**     | 129.7 ± 3.0**        | 214.4 ± 6.9** | 0.65 ± 0.03** | 135.0 ± 5.8**      | 61.4 ± 0.4** |

Data represent mean ± standard deviation (n = 5). *P < 0.05, **P < 0.01: significantly different from Control group.

V/S ratio: Visceral fat weight/Subcutaneous fat weight ratio.
Increased significantly in the pioglitazone 10 mg/kg group, and the relative liver weight in the pioglitazone 10 mg/kg group showed a tendency to decrease (Table 2).

Discussion

The pathogenesis of type 2 diabetes is heterogeneous, and hyperglycemia and dyslipidemia in these patients are caused by defects in insulin secretion and/or insulin sensitivity [15,16]. Accordingly, improving both insulin deficiency in pancreatic β cells and enhancing insulin sensitivity are necessary to achieve tight blood glucose control. Numerous drugs have been developed to treat type 2 diabetic patients, e.g. sulfonylureas and dipeptidyl peptidase (DPP)-4 inhibitors to improve insulin deficiency, biguanides and thiazolidinedione-based agents to enhance insulin sensitivity [15,17]. The most

Table 2: Effects of pioglitazone on organ weights in female SDT fatty rats at 13 weeks of age.

|                  | Absolute kidney weight (mg) | Absolute liver weight (g) | Relative kidney weight (mg/g) | Relative liver weight (g/g) |
|------------------|-----------------------------|---------------------------|-------------------------------|---------------------------|
| Control group    | 3754 ± 194                  | 29.1 ± 11.0               | 7.882 ± 0.922                 | 0.0594 ± 0.0172           |
| Pioglitazone 1 mg/kg group | 3442 ± 361                  | 27.1 ± 9.8                | 7.121 ± 1.083                 | 0.0541 ± 0.0126           |
| Pioglitazone 10 mg/kg group | 3083 ± 175*                 | 26.3 ± 3.4                | 4.805 ± 0.072*                | 0.0411 ± 0.0056           |

Data represent mean ± standard deviation (n = 5). *P < 0.05, **P < 0.01: significantly different from Control group.
The increase in body weight with pioglitazone treatment was supported by results in CT analysis. Pioglitazone treatment increased both visceral and subcutaneous fat weights and, in particular, an increase in subcutaneous fat weight was noted (rate of increase compared with the control group: 84% increase in subcutaneous fat vs. 39% increase in visceral fat); however, the V/S ratio decreased. Pioglitazone reportedly increases fat masses by promoting adipocyte differentiation via PPAR γ activation [21,22]. It is considered that the increase in body weight with pioglitazone treatment is caused by retention of fluid and increase in fat mass, and the increase in fat weight is induced by activating adipocyte differentiation.

The vacuolation of islets and the tubular epithelium are considered to be caused by glycogen accumulation associated with sustained hyperglycemia in SDT fatty rats, and these changes improved with glycemic control observed after pioglitazone treatment. The HbA1c level in the pioglitazone group also decreased. The histopathological abnormalities including irregular boundaries and atrophy in islets improved with glycemic control, and the improvements are considered to induce increases in blood insulin levels. The tubular dilation in the pioglitazone groups is considered to be inhibited by decrease of urine volume with an improvement of hyperglycemia. Moreover, nephromegaly is reportedly observed in type 2 diabetic rat models [23]. The kidney weights of pioglitazone-treated rats were reduced by satisfactory glycemic control. Decreases of lean body mass in the pioglitazone groups may be related with the decreases of organ weights, such as kidney and liver.

Female SDT fatty rats reportedly present with NASH-like hepatic lesions, such as inflammation and fibrosis [24]. In this study, pioglitazone treatment for 6 weeks improved fatty livers in SDT fatty rats, and longer treatment is expected to also improve NASH-like lesions in female SDT fatty rats.

In female SDT fatty rats, the repeated administration of pioglitazone led to improvements in glucose/lipid metabolic abnormalities and pathological lesions in the pancreas, kidney, and liver. However, pioglitazone treatment also induced increases in body weight. The female SDT fatty rat is useful for the development of new anti-diabetic drugs which show potential to enhance insulin sensitivity.

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