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Pioglitazone-induced body weight gain is prevented by combined administration with the lipoprotein lipase activator NO-1886

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ABSTRACT

Pioglitazone improves insulin resistance in diabetics but often causes body weight gain. The lipoprotein lipase activator NO-1886 has been shown to exert both anti-obesity and anti-insulin-resistance effects. In this study, we investigated the effect of the combined administration of pioglitazone with NO-1886 (pioglitazone + NO-1886) in preventing body weight gain in insulin-resistant, high-fat fed rats. The rats were fed a standard or high-fat diet for 16 weeks. The high-fat fed rats were randomized at week 9 into 4 groups (i.e., control, pioglitazone (30 mg/kg/day), NO-1886 (100 mg/kg/day), and pioglitazone + NO-1886 (30 and 100 mg/kg/day, respectively)). The high-fat fed control rats developed obesity and insulin resistance. After 7 weeks of drug treatment, pioglitazone + NO-1886 was found to prevent the body weight gain caused by pioglitazone alone (pioglitazone + NO-1886: $\Delta 76.0 \pm 16.8$ g vs. pioglitazone: $\Delta 127.8 \pm 39.5$ g, $P<0.05$) and to increase glucose infusion rate during insulin clamp, compared with the results in the high-fat fed control group. No differences in plasma nonesterified fatty acid, leptin, adiponectin, glucose, or insulin levels were observed between the pioglitazone + NO-1886 and the pioglitazone-alone groups. However, plasma total cholesterol and HDL-cholesterol levels were significantly increased and plasma triglyceride levels were slightly decreased in the pioglitazone + NO-1886 group, compared with the values in the pioglitazone-alone group. In summary, the combined administration of pioglitazone and NO-1886 prevented the pioglitazone-induced body weight gains and ameliorated insulin resistance observed in high-fat fed rats. These results indicate that combined therapy with pioglitazone and NO-1886 may be beneficial for the treatment of type 2 diabetes.

1. Introduction

The antidiabetic agent pioglitazone is thought to promote the differentiation of adipocytes, convert large-type hypertrophic adipocytes into small-type adipocytes, and to increase insulin activity through peroxisome proliferator-activated receptor-γ (PPARγ) activation (de Souza et al., 2001; Spiegelman, 1998). In addition, thiazolidinediones have also been shown to improve the serum levels of several adipocytokines, such as adiponectin and TNF-α, in type 2 diabetic patients (Miyazaki and DeFronzo, 2008; Oz et al., 2008). However, as a result of the enhanced adipocyte differentiation, pioglitazone treatment has been shown to be associated with body weight gain in obese animals and type 2 diabetic patients (Boden and Zhang, 2006; de Souza et al., 2001; Hermansen and Mortensen, 2007; Miyazaki and DeFronzo, 2008). As the mechanism underlying the body weight gain, Hallakou et al. (1997) explained that pioglitazone stimulated the expression of genes involved in lipid metabolism and induced a large increase in glucose utilization in the adipose tissue. Obesity aggravates diabetes and promotes cardiovascular diseases and atherosclerosis (Allison et al., 1999; Fontaine et al., 2003; Kadowaki and Yamauchi, 2005), and the body-weight-increasing action of pioglitazone is a disadvantage in diabetic patients. On the other hand, the lipoprotein lipase (LPL) activator

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NO-1886 (Kusunoki et al., 2002; Tsutsumi et al., 1993, 1995) is known to improve both obesity and insulin resistance in obese animals (Hara et al., 1998; Kusunoki et al., 2000).

In this study, we investigated the effect of the simultaneous administration of pioglitazone, which induces a body weight gain, and NO-1886, which has an anti-obesity action, on the body weight and insulin resistance of obese rats.

2. Material and methods

2.1. Materials

NO-1886 (4-diethoxyphosphorylmethyl-N-(4-bromo-2-cyanophenyl) benzamide; ibrolipim), was obtained from Otsuka Pharmaceutical Factory, Inc. (Naruto, Tokushima, Japan). Pioglitazone was obtained from Takeda Chemicals Industries, Ltd. (Osaka, Japan). All other chemicals used in this study were high-grade, commercially available products.

2.2. Animal experiments

Male SD rats aged 6–7 weeks old and weighing 180–200 g, were obtained from Japan SLC, Inc. (Shizuoka, Japan). The animals were maintained under a 12-h light–dark cycle (lights on from 7:00 am to 7:00 pm) at a constant temperature of 23 ± 2 °C.

Rats were assigned to one of 5 groups according to the diet that they were fed for 16 weeks: (a) normal group that was fed standard laboratory chow (MF; Oriental Yeast Co., Ltd., Tokyo, Japan; 6% of caloric intake as fat and 65% as carbohydrate); (b) high-fat diet group that was fed a high-fat diet prepared by mixing safflower oil into standard laboratory chow (approximately 60% of the caloric intake as fat), as previously described (Kusunoki et al., 1993; Storlien et al., 1987); (c) pioglitazone group that was fed a high-fat diet containing pioglitazone; (d) NO-1886 group that was fed a high-fat diet containing NO-1886; (e) pioglitazone + NO-1886 group that was fed a high-fat diet containing pioglitazone and NO-1886. The dose of pioglitazone was 30 mg/kg/day, and the dose of NO-1886 was 100 mg/kg/day. The pioglitazone, NO-1886, and pioglitazone + NO-1886 groups were fed the high-fat diet from week 0 to 9, and were then treated with the drug(s) added to the high-fat diet from week 9 to 16 (Table 1). The animals were given free access to food and tap water. The calorie consumption and body weight were recorded weekly. At the end of the experimental period, the animals were sacrificed by exsanguination under pentobarbital sodium anesthesia after overnight fast. Blood samples were collected from the posterior vena cava for the measurement of the plasma lipid levels (total cholesterol, HDL-cholesterol (HDL-C), triglyceride and nonesterified fatty acid), leptin, adiponectin, glucose and insulin.

2.3. Euglycemic hyperinsulinemic clamp studies

Insulin action was assessed in each of the five groups at the end of the 16-week feeding period. Euglycemic glucose clamp studies (Kraegen et al., 1983; Storlien et al., 1987) were performed after 12-h overnight fast. The clamps were performed under anesthesia, with the body temperature maintained at 37 °C. The jugular and carotid cannulae were inserted under pentobarbital sodium anesthesia (50 mg/kg body weight). An extension tubing was attached to the jugular vein with an adapter, so as to allow the glucose and insulin to be infused simultaneously into the jugular vein. The carotid catheter was used for blood sampling. After a 30-min basal period, human regular insulin (Novolin R; Novo Nordisk A/S, Bagsværd, Denmark) was continuously infused at a rate of 60 pmol/kg/min throughout the study. Blood samples were drawn at 10-min intervals for the immediate measurement of the plasma glucose level, which was kept constant at approximately 4.5 mmol/l by the variable infusion of 10% dextrose solution. A steady state was achieved within 60 to 100 min in all the rats, and the steady-state glucose infusion rate (GIR, glucose mg/kg/min) was obtained over the next 30 min. All the animal experiments were conducted with the approval of the local Animal Ethics Committee of Aichi Medical University.

Table 1
Characteristics of experimental animals.

Group	Diet (treatment)	Treatment period
Normal	Standard chow	–
High-fat diet		
Control	High-fat diet	–
Pio	High-fat diet + pioglitazone	7 weeks (week 9–16)
NO-1886	High-fat diet + NO-1886	7 weeks (week 9–16)
Pio + NO-1886	High-fat diet + pioglitazone + NO-1886	7 weeks (week 9–16)

Dose of pioglitazone: 30 mg/kg/day; NO-1886: 100 mg/kg/day. Pio, pioglitazone-administered group; NO-1886, NO-1886-administered group; Pio + NO-1886, group treated with Pio and NO-1886 in combination.

2.4. Analytical methods

The plasma lipid levels were assayed using standard kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The glucose level was measured on a YSI 2300 glucose analyzer (YSI Incorporated Life Sciences, OH, USA), and the insulin level was measured using a conventional enzyme immunoassay with the Glazyme insulin-EIA test kit (Wako Pure Chemical Industries, Ltd.). The leptin and adiponectin levels were assayed with the Rat Leptin ELISA Kit (Morinaga Institute of Biological Science, Inc., Kanagawa, Japan) and the Human Adiponectin ELISA Kit for Total and Multimers (Sekisui Medical Co., Ltd., Tokyo, Japan), respectively.

2.5. Statistical analysis

The results were expressed as mean ± S.D. Comparisons among the groups were analyzed for statistical significance by two-sided unpaired Student's *t*-test and one-way analysis of variance, followed by the Tukey's test for multiple comparisons. *P* values less than 0.05 were considered significant.

3. Results

3.1. Body weight, visceral fat weight, and food consumption

The body weight in the high-fat diet group was generally higher than that in the normal group. The body weight in the pioglitazone group was significantly higher than that in the normal diet group over a period of 7 weeks during the study period (week 9 to week 16). In contrast, the body weight in the NO-1886 group was significantly lower than that in the pioglitazone group during the last 4 weeks (week 12 to week 16). The body weight in the pioglitazone + NO-1886 group was significantly lower than that in the pioglitazone group at week 16 (Fig. 1A). The extents of the body weight changes after the start of drug treatment are shown in Fig. 1B. Reflecting the body weight trends, the extent of the change in the pioglitazone group was generally higher than those in the other 4 groups, and the change was significant between the pioglitazone and pioglitazone + NO-1886 groups at the end of the study period (week 16).

In the high-fat diet, pioglitazone and pioglitazone + NO-1886 groups, the visceral fat weights were significantly higher than that in the normal diet group. These results indicated a 1.8-fold higher visceral fat weight in the high-fat diet group than in the normal diet group. The visceral fat weight was 24.6% higher in the pioglitazone group than in the high-fat diet group (not significant). In the NO-1886 group, on the other hand, the visceral fat weight was significantly lower than that in the pioglitazone group (Fig. 2).