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NO-1886 (ibrolipim), a lipoprotein lipase activator, increases the expression of uncoupling protein 3 in skeletal muscle and suppresses fat accumulation in high-fat diet–induced obesity in rats

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Abstract

Although the lipoprotein lipase (LPL) activator NO-1886 shows antiobesity effects in high-fat–induced obese animals, the mechanism remains unclear. To clarify the mechanism, we studied the effects of NO-1886 on the expression of uncoupling protein (UCP) 1, UCP2, and UCP3 in rats. NO-1886 was mixed with a high-fat chow to supply a dose of 100 mg/kg to 8-month-old male Sprague-Dawley rats. The animals were fed the high-fat chow for 8 weeks. At the end of the administration period, brown adipose tissue (BAT), mesenteric fat, and soleus muscle were collected and levels of UCP1, UCP2, and UCP3 messenger RNA (mRNA) were determined. NO-1886 suppressed the body weight increase seen in the high-fat control group after the 8-week administration (585 ± 39 vs 657 ± 66 g, $P < .05$). NO-1886 also suppressed fat accumulation in visceral (46.9 ± 10.4 vs 73.7 ± 14.5 g, $P < .01$) and subcutaneous (43.1 ± 18.1 vs 68.9 ± 18.8 g, $P < .05$) tissues and increased the levels of plasma total cholesterol and high-density lipoprotein cholesterol in comparison to the high-fat control group. In contrast, NO-1886 decreased the levels of plasma triglycerides, nonesterified free fatty acid, glucose, and insulin. NO-1886 increased LPL activity in soleus muscle (0.082 ± 0.013 vs 0.061 ± 0.016 μmol of free fatty acid per minute per gram of tissue, $P < .05$). NO-1886 increased the expression of UCP3 mRNA in soleus muscle 3.14-fold ($P < .01$) compared with the high-fat control group without affecting the levels of UCP3 in mesenteric adipose tissue and BAT. In addition, NO-1886 did not affect the expression of UCP1 and UCP2 in BAT, mesenteric adipose tissue, and soleus muscle. In conclusion, NO-1886 increased the expression of UCP3 mRNA and LPL activity only in skeletal muscle. Therefore, a possible mechanism for NO-1886's antiobesity effects in rats may be the enhancement of LPL activity in skeletal muscle and the accompanying increase in UCP3 expression.

1. Introduction

We have previously reported that the lipoprotein lipase (LPL) activator NO-1886 suppresses subcutaneous and visceral fat accumulation and shows antiobesity effects in high-fat–induced obese rats and pigs [1,2]. We also suggested that the antiobesity effects of NO-1886 might be the result of the increase in skeletal muscle LPL activity because NO-1886 had no effect on adipose tissue LPL activity [1]. However, this proposed mechanism remained unclear. Some investigators have suggested that increasing LPL activity in adipose tissue results in increased fat accumulation [3,4], whereas others have suggested that increasing LPL activity in skeletal muscle results in decreased fat accumulation [5-7]. It has long been known that uncoupling proteins (UCPs) are responsible for facultative thermogenesis in rodents. UCPs play an important role in energy metabolism and obesity [8,9]. UCP1 expression is restricted to brown adipose tissue (BAT), UCP2 is widely expressed, and UCP3 is found mainly in

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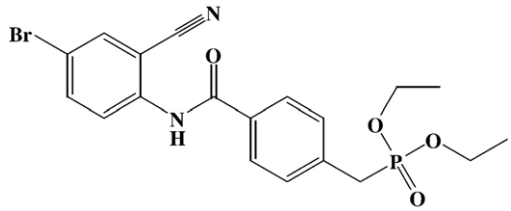

Fig. 1. Chemical structure of NO-1886.

skeletal muscle [10]. To clarify the relationship between skeletal muscle LPL activity and the antiobesity effects of NO-1886, we studied the effects of NO-1886 on the expression of UCP1, UCP2, and UCP3 in rats.

2. Materials and methods

2.1. Materials

Agent NO-1886 (ibrolipim) [4-diethoxyphosphoryl-methyl-*N*-(4-bromo-2-cyanophenyl)benzamide, CAS 133208-93-2; lot C99H74SM] was synthesized in the New Drug Research Laboratory of Otsuka Pharmaceutical Factory, Tokushima, Japan. Glycerol tri[1-^{14}C]oleate (2.2 GBq/mmol) was obtained from Amersham, Cardiff, UK. The chemical structure is shown in Fig. 1. All other chemicals used were high-grade commercially available products.

2.2. Animal experiments

Male Sprague-Dawley rats weighing 500 to 600 g at the age of 8 months were obtained from Japan SLC, Shizuoka, Japan. The animals were maintained under a 12-h light-dark cycle (light cycle from 7:00 AM to 7:00 PM) at a constant temperature of 23°C ± 2°C. Rats were fed for 8 weeks with either high-fat chow (26.7% safflower oil in standard laboratory chow; CRF-1, Oriental Yeast, Tokyo, Japan) containing NO-1886 (NO-1886 group 20.17 kJ/g [4.82 kcal/g]), high-fat chow (high-fat group, 20.17 kJ/g [4.82 kcal/g]), or standard chow (low-fat group, 15.06 kJ/g [3.6 kcal/g]). The rats were housed 1 per cage. The rats were allocated to the high-fat group, NO-1886 group, or low-fat group based on baseline body weight and were given free access to food and tap water. Food consumption and body weight were recorded every 4 weeks. At the end of the experimental period and after a 12-hour overnight fast, the animals were killed by exsanguination under sodium pentobarbital anesthesia. Blood samples were collected from the posterior vena cava for lipid, glucose, and insulin measurements. Visceral fat and subcutaneous fat were removed and weighed. Soleus hind limb muscle, mesenteric white adipose tissue, and scapula BAT were obtained with tongs and maintained in liquid nitrogen for measurement of LPL activity and UCP messenger RNA (mRNA).

2.3. Analytical methods

Plasma total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, nonesterified free fatty acid (NEFA), and glucose were determined by conventional enzymatic methods. The cholesterol C-test Wako (Wako Pure Chemical Industries, Osaka, Japan) was used for cholesterol, the Nescote HDL-C kit N (Nippon Shoji, Osaka Japan) for HDL-C, the triglyceride G-test Wako (Wako Pure Chemical Industries) for triglycerides, the NEFA C-test Wako (Wako Pure Chemical Industries) for NEFA, and the glucose CII test Wako for glucose. Insulin was determined by conventional enzyme immunoassay, with the use of the Glazyme insulin-EIA test (Wako Pure Chemical Industries).

2.4. Tissue LPL activity

Soleus heparin-released LPL activity was measured as reported previously [11,12]. A specimen of muscle was homogenized in 50 mmol/L NH_4Cl buffer (pH 8.5) and incubated with buffer containing heparin for 60 minutes at 0°C. The suspension was then centrifuged, and the supernatant was used to measure heparin-released LPL activity. Adipose tissue heparin-released LPL activity was measured

Table 1
Food intakes of low-fat–fed rats and NO-1886–treated high-fat–fed rats

Group	n	Food intake (g)			Food intake (kcal [kJ])		
		Before	4 wk	8 wk	Before	4 wk	8 wk
Low fat[a]	6	17.6 ± 3.6	18.0 ± 4.9*	18.4 ± 2.6*	63.4 ± 12.9 [265.3 ± 54.0]	64.8 ± 17.6 [271.1 ± 73.6]	66.2 ± 9.4 [277.0 ± 39.3]
High fat[b]							
Control	6	18.5 ± 4.9	13.7 ± 1.5	14.3 ± 1.5	66.6 ± 17.6 [278.6 ± 73.6]	66.0 ± 7.2 [278.6 ± 30.1]	68.9 ± 7.2 [288.3 ± 30.1]
NO-1886	6	19.1 ± 2.6	14.0 ± 1.7	14.8 ± 2.8	68.7 ± 9.4 [287.4 ± 39.3]	67.5 ± 8.1 [282.4 ± 33.9]	71.3 ± 13.5 [298.3 ± 56.5]

Data are expressed as means ± SD.
Before indicates before starting high-fat diet.
[a] Low-fat chow: 15.06 kJ/g (3.6 kcal/g).
[b] High-fat chow: 20.17 kJ/g (4.82 kcal/g).
* $P < .05$, significantly different from high-fat control group.