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Anti-Diabetic Effects of DA-11004, a Synthetic IDPc Inhibitor in High Fat High Sucrose Diet-Fed C57BL/6J Mice

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DA-11004 is a synthetic, potent NADP-dependent isocitrate dehydrogenase (IDPc) inhibitor where IC_{50} for IDPc is 1.49 μM. The purpose of this study was to evaluate the effects of DA-11004 on the high fat high sucrose (HF)-induced obesity in male C57BL/6J mice. After completing a 8-week period of experimentation, the mice were sacrificed 1hr after the last DA-11004 treatment and their blood, liver, and adipose tissues (epididymal and retroperitoneal fat) were collected. There was a significant difference in the pattern of increasing body weight between the HF control and the DA-11004 group. In the DA-11004 (100 mg/kg) treated group the increase in body weight significantly declined and a content of epididymal fat and retroperitoneal fat was also significantly decreased as opposed to the HF control. DA-11004 (100 mg/kg) inhibited the IDPc activity, and thus, NADPH levels in plasma and the levels of free fatty acid (FFA) or glucose in plasma were less than the levels of the HF control group. In conclusion, DA-11004 inhibited the fatty acid synthesis in adipose tissues *via* IDPc inhibition, and it decreased the plasma glucose levels and FFA in HF diet-induced obesity of C57BL/6J mice.

Key words: DA-11004, Obesity, Diabetes, NADP-dependent Isocitrate dehydrogenase, NADPH, High fat high sucrose

INTRODUCTION

Obesity is a major risk factor for the development of type 2 diabetes mellitus and is associated with pathologic states, such as hypertension, dyslipidaemia, and atherosclerosis (Reaven, 1991). The fatty acid composition of diets is important in the cause of type 2 diabetes. Numerous studies have shown that a high-fat diet directly relates to the development of obesity, and high-fat feeding can induce syndromes of glucose intolerance and/or insulin resistance in human and animals (Storlien *et al.*, 1996).

A relationship between high fat diets, obesity, and type 2 diabetes mellitus has also been found in rodents. One animal model that is particularly susceptible to dietary effects is the C57BL/6J mouse. This animal will develop obesity and type 2 diabetes mellitus when fed a high fat diet, and it closely resembles common forms of the human disease after developing obesity (Surwit *et al.*, 1988; Surwit *et al.*, 1991).

Fatty acids are synthesized through a common biochemical pathway in all organisms and are stored as a form of triglycerides. NADPH has an important role as a cofactor in fatty acid synthesis. Many enzymes in central biosynthetic pathways require reducing equivalents in the form of NADPH, whereas the enzymatic sources of biosynthetic reducing equivalents are believed to be limited in number. The oxidative branch of the hexose monophosphate shunt or pentose phosphate pathway is generally accepted to be the major cellular source of NADPH (Minard *et al.*, 1998).

NADP-dependent isocitrate dehydrogenase (IDPc) is involved in obesity through the production of NADPH, an important cofactor for fatty acid synthesis. IDPc provides NADPH for the reduction of double bonds (van Roermund

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et al., 1998).

In this study, we evaluated the effects of DA-11004, a synthetic IDPc inhibitor, in HF diet-fed C57BL/6J mice.

MATERIALS AND METHODS

Materials

DA-11004, or 4-aminomethyl-1,2-naphthoquinone, was synthesized from Dong-A Pharm. Co.Ltd (Korea); high fat high sucrose diet (20% fat, 45% sucrose) was purchased from Bio-serv (Frenchtown, NJ); and Tris-HCl, EDTA, phenazine ethosulfate, MOPS, triethanolamine, $NADP^+$, isocitrate, magnesium chloride, rotenone and other reagents were purchased from Sigma (St Louis, MO).

Animals and diets

Male C57BL/6J mice were obtained from Bio-Genomics Co.Ltd (Seoul, Korea). The mice were kept in a temperature- and humidity-controlled room with a 12:12-h light: dark cycle. Throughout the study, the mice were provided standard laboratory chow (fat ~7%, sucrose 2%, corn starch 34%) or high fat high sucrose (fat: 20%, sucrose 40%) *ad libitum*. Their body weight was recorded twice a week, and the drug (po) was administrated daily for 8 weeks. On the final day of the experiments, the mice were killed by exsanguinations under ether anesthesia, and the blood was collected while the liver, epididymal and retroperitoneal fat were immediately excised for weighing.

Assay of IDPc enzyme activity and NADPH

Isocitrate dehydrogenase (IDPc) catalyzes the oxidative decarboxylation of isocitrate into 2-oxoglutarate by NAD- or NADP-dependent dehydrogenase (Raunio *et al.*, 1985). After a protein assay, samples (10 μL) were added to the reaction solution (final volume, 1 mL) containing the IDPc buffer (50 mM MOPS, pH 7.2), 35.5 mM triethanolamine (pH 7.2), 2 mM $NADP^+$, 2 mM $MgCl_2$, 5 mM isocitrate, and 1 μg/mL rotenone and were measured for absorbance at 340 nM for 3 min at 0, 60 min. The activity of IDPc was expressed as the Unit (U). 1 U is defined as the enzyme activity of producing 1 μM NADPH/min at 25°C.

In order to determine NADPH levels, samples (100 μL) were added to the reaction solution containing 0.1 M Tris-HCl (pH 8.0), 5mM EDTA, 2 mM phenazine ethosulfate, 0.5 mM MTT and were measured for absorbance at 570 nm for 3 min. Standard NADPH (Sigma, St Louis, MO) was diluted as 0, 50, 100, 200, 400 μM and its absorbance was measured at 570 nm for 3 min. The content of plasma NADPH was calculated from the standard NADPH absorbance.

Plasma glucose, triglycerides, non-esterified fatty acid analysis

The plasma glucose (GOD/POD Enzymatic method) and triglycerides (Enzymatic GPO/Trinder method) were determined using the selective chemistry analyzer (Konelab 60i, Finland). Plasma free fatty acids (FFA) were measured using the acetyl CoA synthase-acetyl CoA oxidase (ACS-ACO) enzyme assay kits (Azwell, Osaka).

Data analysis

Data was expressed as the mean±S.E. Statistical differences between means were determined by the Student's *t*-test.

RESULTS

The effects of DA-11004 on body weight and tissue weight in high fat high sucrose diet-fed male C57BL/6J mice

The body weight of the high fat high sucrose C57BL/6J mice was 22.5% higher at 8 weeks than that of the normal chow-fed C57BL/6J mice; however, DA-11004 (100 mg/kg, po) significantly inhibited the increase of body weight in fat-fed C57BL/6J mice (Table I, Fig. 1). In the DA-11004 treated group, the contents of food consumption were decreased when compared to the HF control group (data not shown). The contents of epididymal fat and retroperitoneal fat were 98% and 112% higher when compared to the normal chow-fed C57BL/6J mice, respectively. However, in the DA-11004 (100 mg/kg) treated C57BL/6J mice, fat contents were similar to those of normal chow-fed C57BL/6J mice. The liver weight was not significantly different between the fat-fed control and the DA-11004 treated group (Table I).

Table I. The effect of DA-11004 on body weight, liver, and adipose tissues in high fat high sucrose (HF) C57BL/6 mice

	Body weight (g)	Liver (g)	Epididymal fat (g)	Retroperitoneal fat (g)
Normal	25.6 ± 0.31	0.97 ± 0.02	0.70 ± 0.07	0.33 ± 0.04
HF control	31.4 ± 0.68**	0.96 ± 0.02	1.39 ± 0.13**	0.69 ± 0.04**
DA-11004 (30 mg/kg)	30.0 ± 0.83**	0.99 ± 0.04	1.26 ± 0.16*	0.65 ± 0.07**
DA-11004 (100 mg/kg)	24.1 ± 0.55##	0.90 ± 0.03	0.63 ± 0.05##	0.30 ± 0.03##

Data are expressed as the mean±SE (n=7-10). *P<0.05, **P<0.01 vs. normal, ##P<0.01 vs. HF control.

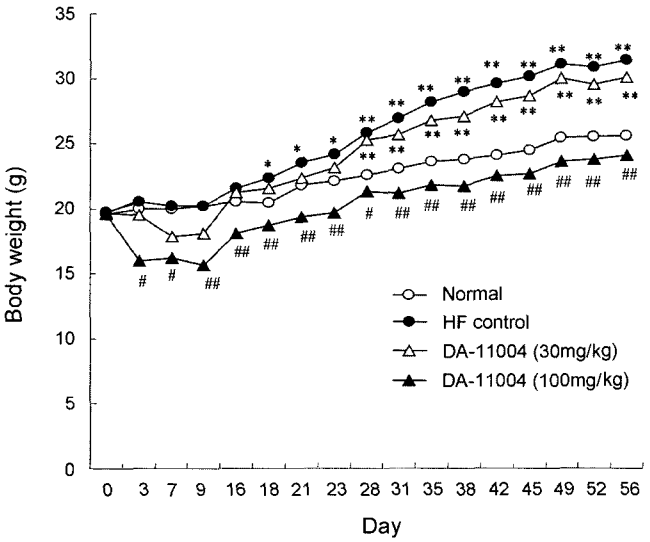


Fig. 1. The effects of the 8 week treatment with different selected doses of DA-11004 on body weight in high fat, high sucrose C57BL/6 mice. DA-11004 (100 mg/kg) inhibited the increase of body weight in the fat-fed C57BL/6J mice. Animals were treated with DA-11004 during the 8 week *P<0.05, **P<0.01 vs. normal; #P<0.05, ##P<0.01 vs. HF control (n=10).

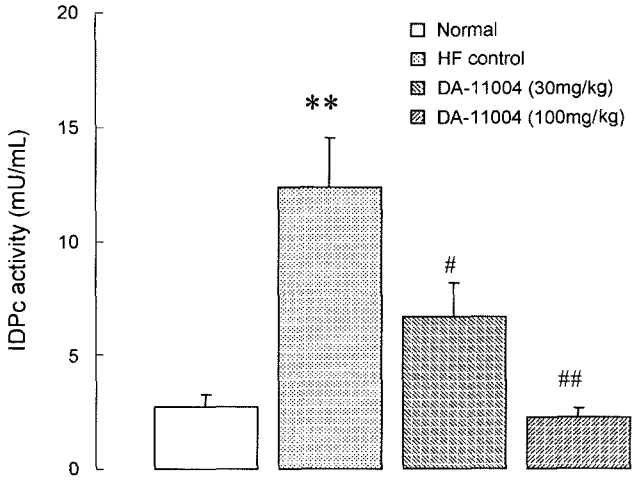
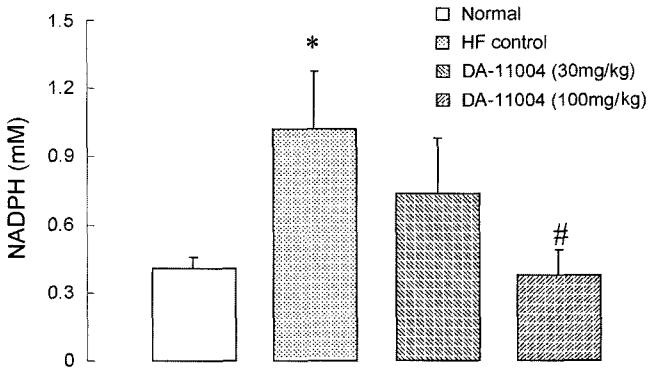


Fig. 2. The effects of DA-11004 on the IDPc activity in fat-fed C57BL/6 mice in plasma. DA-11004 dose-dependently inhibited the IDPc activity when compared to the HF control. Data were expressed as the mean ± SE. **P<0.01 vs. normal; #P<0.05, ##P<0.01 vs. HF control (n=7-8).

NADP-dependent isocitrate dehydrogenase (IDPc) activity and NADPH levels in plasma

We investigated the role of DA-11004 on IDPc enzyme activity in fat-fed C57BL/6J mice. In the fat-fed control, the IDPc activity was 6 times higher than that of the compared with normal chow-fed control; however, DA-11004 inhibited the IDPc activity dose-dependently (Fig. 2). IDPc enzymes provide NADPH for the reduction of double bonds at even positions. Thus, we measured the NADPH



* P<0.05 vs. Normal; #P<0.05 vs. HF

Fig. 3. The effects of DA-11004 on the NADPH levels in the fat-fed C57BL/6 mice plasma. DA-11004 (100 mg/kg) inhibited the production of NADPH levels when compared to the HF control. Data were expressed as the mean±SE. *P<0.05 vs. normal; #P<0.05 vs. HF control (n=7-8).

Table II. Plasma parameters for C57BL/6 mice treated with DA-11004

	Glucose (mg/dL)	Triglycerides (mg/dL)	Total cholesterol (mg/dL)
Normal	167.4± 5.2	89.4±7.1	96.0±1.4
HF control	195.0± 7.4*	90.6±5.7	97.8±1.5
DA-11004 (30 mg/kg)	195.2±12.4*	75.1±0.8	99.1±0.8
DA-11004 (100 mg/kg)	169.3± 7.9#	63.2±2.3##	105.4±2.4

Data are expressed as the mean ± SE (n=7-10). *P<0.05 vs. normal; #P<0.05, ##P<0.01 vs. HF control.

levels in the plasma. In the fat-fed control, NADPH levels was increased 2-3 times when compared to the normal chow-fed control, but DA-11004 decreased the NADPH levels dose-dependently when compared with the fat-fed control (Fig. 3).

Plasma glucose, triglycerides and free fatty acids

In the fat-fed control, plasma glucose levels were 16% higher when compared with the normal chow-fed control (Table II). However, in the DA-11004 (100 mg/kg) treated group, the plasma glucose level was similar to those of the normal chow-fed control, and triglycerides levels were 43% lower when compared with the fat-fed control. In the fat-fed control, the free fatty acid level was 1158±63 (μEq/L), but DA-11004 (30 mg/kg, 100 mg/kg) inhibited the FFA production in plasma from 1158 63 to 979±23, 955±39 (μEq/L), respectively (Fig. 4).

DISCUSSION

Obesity has developed into a significant health problem in westernized societies over the past 20 years due to its

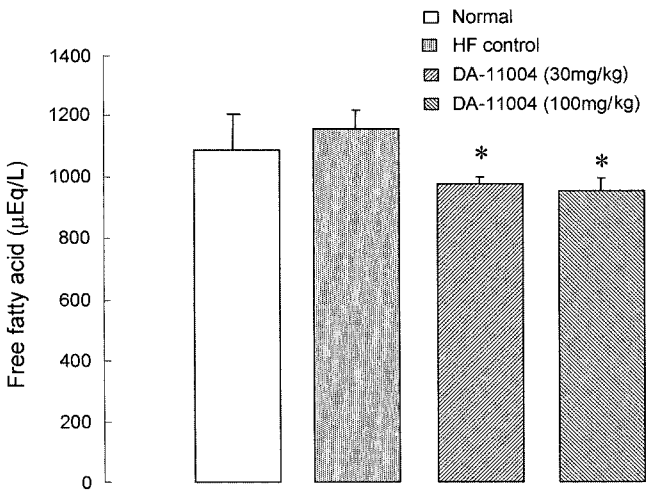


Fig. 4. The effect of DA-11004 on the free fatty acid (FFA) levels in the fat-fed C57BL/6 mice plasma. DA-11004 reduced the FFA levels when compared to the HF control. Data were expressed as the mean±SE. #P<0.05 vs. HF control (n=7-8).

association with various chronic diseases such as non-insulin-dependent diabetes, cardiovascular disease, and cancer (Flegal *et al.*, 1998; Kahn and Flier, 2000). The increased prevalence of obesity has been attributed to the increased availability and consumption of fat-rich foods and reduced physical activity (Spiegelman and Flier, 2001). This has led many researchers to develop animal models that will allow for the study of mechanisms by which diet-induced obesity contributes to various disease states. One animal model that is particularly susceptible to dietary effects is the C57BL/6J mouse. The C57BL/6J mouse has previously been shown to be a good model for studying diet-induced obesity and diabetes. It develops obesity, insulin resistance, and hyperlipidemia, which resembles the human type 2 diabetes after feeding on a western-type, high-fat diet (Surwit *et al.*, 1988; Surwit *et al.*, 1991; West *et al.*, 1992).

NADP-linked isocitrate dehydrogenase (IDPc) is the major source of NADPH (Bruinenberg *et al.*, 1983) and NADPH is produced by IDPc and, as a cofactor, is needed in process of fat synthesis process (van Roermund *et al.*, 1998). Reducing equivalents in the form of NADPH are essential for many enzymatic steps involved in the biosynthesis of cellular macromolecules. NADPH is also the essential cofactor for glutathione- and thioredoxin-dependent enzymes that constitute major cellular defenses against oxidative damage (Halliwell, 1994). In eukaryotic cells, the majority of these NADPH-dependent reactions occur in the cytosol.

The predominant source of energy for a resting skeletal muscle is fatty acid oxidation. Glucose and ketone body metabolism also contribute in varying degrees, depending on the activity and metabolic state of the muscle. However, in diabetes there is a diminished contribution of glucose and ketone bodies to the skeletal muscle's energy metabolism. Hence, there is an increased demand on the muscle for fatty acid oxidation to fill its energy needs (Yechoor *et al.*, 2002). β-Oxidation includes multiple cycles leading to the formation of acetyl-CoA, with the first step of each cycle consisting of dehydrogenation, followed by hydration, a second dehydrogenation, and finally thiolysis. In peroxisomal β-oxidation of polyunsaturated fatty acids in *Saccharomyces cerevisiae*, IDPc provides NADPH for the reduction of double bonds at even positions (van Roermund *et al.*, 1998).

DA-11004 is a synthetic inhibitor of IDPc, where IC_{50} for IDPc is 1.49 μM (0.9 μg/mL). We found that DA-11004 reduced the increases in body weight and fat weight in fat-fed C57BL/6J mice, which suggested that DA-11004 inhibited fat-fed obesity through the inhibition of fat synthesis. We found that the plasma glucose and triglycerides levels were decreased in DA-11004 treated groups when compared to the fat-fed control. Then, we measured the IDPc activity and NADPH levels in plasma, and both levels were more declined than the levels of the fat-fed control. It suggested that DA-11004 inhibited the NADPH production *via* the inhibition of IDPc. Free fatty acids (FFA) are speculated to play a role in the development of diabetes. FFA increases glucose output from the liver and decreases glucose uptake in skeletal muscles (Boden, 1997). In islets of animal models of diabetes, FFA impaired the glucose-stimulated insulin secretion *in vivo* and *in vitro* (Elks, 1993; Zhou and Grill, 1994). It has been reported that high fat and high sucrose feeding increased plasma triglyceride, free fatty acid (FFA), and glucose levels and decreased HDL-C level (Yin *et al.*, 2003). When DA-11004 was treated in the fat-fed C57BL/6J mice, the plasma FFA levels were significantly decreased when compared with the levels of the fat-fed control.

In summary, DA-11004 inhibited the fatty acid synthesis in adipose tissues via IDPc inhibition and decreased the plasma glucose levels and FFA in C57BL/6 mice that were obese through HF diet-induction.

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