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A novel coenzyme A:diacylglycerol acyltransferase 1 inhibitor stimulates lipid metabolism in muscle and lowers weight in animal models of obesity

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ABSTRACT

Obesity is characterized by the accumulation of triacylglycerol in adipocytes. Coenzyme A:diacylglycerol acyltransferase 1 (DGAT1) is one of two known DGAT enzymes that catalyze the final and only committed step in triacylglycerol synthesis. In this report, we describe the pharmacological effects of a novel selective DGAT1 inhibitor, Compound-A. This compound inhibited triacylglycerol synthesis in both adipocytes and skeletal myotubes, and increased fatty acid oxidation in skeletal myotubes at 1 μM. The repeated administration of Compound-A to diet-induced obese C57BL/6J and genetically obese KKA^{y} mice (3–30 mg/kg for 3–4 weeks) significantly decreased the visceral fat pad weights and the hepatic lipid contents compared to controls without affecting food intake. In addition, fatty acid oxidation in skeletal muscle tissues was increased by the treatment of Compound-A in both mice strains. This is the first report demonstrating that a small synthetic DGAT1 inhibitor increases fatty acid oxidation in skeletal muscle *in vitro* and *ex vivo*. These results suggest that DGAT1 inhibition is a promising therapeutic approach for the treatment of obesity and lipid abnormalities such as hepatic steatosis.

1. Introduction

Acyl-coenzyme A:diacylglycerol acyltransferase (DGAT, EC 2.3.1.20) is an endoplasmic membrane-bound enzyme that catalyzes the final step of triacylglycerol synthesis. There are two known DGAT isozymes, namely, DGAT1 and DGAT2 (Cases et al., 1998, 2001; Oelkers et al., 1998), with different distributions and functions in mammals (Chen et al., 2002; Smith et al., 2000; Stone et al., 2004; Yamazaki et al., 2005). DGAT1-deficient (DGAT1$^{-/-}$) mice have a reduced amount of visceral adipose tissue (Smith et al., 2000), and decreased triacylglycerol content in skeletal muscles (Chen et al., 2002). They are resistant to high-fat diet-induced obesity (Smith et al., 2000) and more sensitive to insulin than syngeneic wild-type (DGTA1$^{+/+}$) mice (Chen et al., 2002). They also showed improvements in glucose, lipid, and energy metabolism and it may partially correlate with altered secretion of adipocytokines such as leptin and adiponectin in the mice (Chen et al., 2003). On the other hand, DGAT2-deficient (DGAT2$^{-/-}$) mice are smaller than wild-type controls and die within a few hours after birth (Stone et al., 2004), and inbred DGAT2-heterozygous (DGAT2$^{+/-}$) mice are not protected from diet-induced obesity (Chen and Farese, 2005). These findings suggest that a selective inhibitor of DGAT1 is potentially useful for the treatment of obesity and/or diabetes and that compounds which selectively or also inhibit DGAT2 would have a narrower therapeutic window. Moreover, since the function and expression pattern of DGAT1 in mice are proposed to be similar to those in humans (Yen et al., 2008), the physiological function of DGAT1 in mice may be pertinent to humans.

Several DGAT inhibitors from natural sources have been reported; however, their inhibitory potency against DGAT isozymes is quite low and their inhibitory activity (IC_{50}) is in the range of only 10–100 μM (Inokoshi et al., 2009). To obtain compounds to investigate whether DGAT1 selective inhibitors would have the highly desired metabolic effects similar to those observed in the knock-out animals, several pharmaceutical companies have pursued medicinal drug discovery programs. As a results of these efforts, synthetic compounds with strong DGAT1 inhibitory activity have been reported to have anti-

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obesity (Zhao et al., 2008; Birch et al., 2009) and serum lipid-lowering actions *in vivo* (King et al., 2009). In this study, we provide the first report that a selective DGAT1 inhibitor, Compound-A, has potent body weight-reducing action characterized by not only the inhibition of triacylglycerol synthesis and absorption but also the stimulation of fatty acid oxidation in skeletal muscle in both diet-induced obese wild-type and genetically obese mice. These results suggest that DGAT1 inhibition is a promising therapeutic approach for the treatment of obesity, diabetes, and hepatic steatosis.

2. Materials and methods

2.1. Chemicals and reagents

Compound-A (*N*-[2-({[1-phenyl-3-(trifluoromethyl)-1*H*-pyrazol-4-yl]carbonyl}amino)ethyl]-6-(2,2,2-trifluoroethoxy)pyridine-3-carboxamide) (see Fig. 1A), was synthesized at Array BioPharma Inc (Boulder, CO, USA) in a medicinal chemistry collaboration with Takeda Pharmaceutical Company Limited (Osaka, Japan) (Nakada et al., 2010). [1-^{14}C]-Oleoyl-CoA, [9, 10(n)-^{3}H]-palmitic acid, and 2-deoxy-D-[2, 6-^{3}H]-glucose were purchased from Amersham Biosciences (Piscataway, NJ, USA). All other chemicals were of standard reagent grade.

2.2. In vitro enzyme assay

Human DGAT1 (hDGAT1: GeneBank accession no. AB057815), mouse DGAT1 (mDGAT1: GeneBank accession no. AB057816) and human DGAT2 (hDGAT2: GeneBank accession no. NM_032564) were cloned into the pFASTBAC1 vectors (Life Technologies, Bethesda, MD, USA) and expressed in Sf9 insect cells using a baculovirus expression system. Sf9 cells were infected, and the membrane fractions were isolated as the enzyme sources as described by Cases et al. (1998). The reaction mixtures of the hDGAT1 and mDGAT1 enzyme assays contained 100 mM Tris–HCl (pH 7.5), 250 mM sucrose, 150 mM $MgCl_2$, 0.01% bovine serum albumin (fatty acid-free), 1% acetone, 25 μM 1,2-dioleoyl-sn-glycerol, and 5 μg protein/ml of the microsomal membrane fraction. For measurement of the inhibitory activities of DGAT1 inhibitors (i.e., Compound-A), serial dilutions of Compound-A (f.c. 10 pM–10 μM) with DMSO were added to the reaction mixture in the final concentration of 0.1% DMSO. The reaction was initiated by addition of 25 μM [1-^{14}C]-oleoyl-CoA in a final volume of 100 μl, and incubated for 15 min at 32 °C, and the reaction was terminated by addition of 300 μl chloroform:methanol (1:2) solvent. After mixing of the reaction mixture, 200 μl phosphate-buffered saline (PBS) was added and mixed, centrifuged (3000×*g*, 3 min), and the lipids extracted in the organic phase were separated by the thin layer chromatography (TLC) with a solvent system of n-hexane:diethyl ether:ethyl acetate:acetic acid (74:15:15:1). hDGAT2 enzyme assay was carried out in the same way as DGAT1 expect for using 20 mM $MgCl_2$ instead of 150 mM $MgCl_2$. The radioactivity of synthesized [^{14}C]-triacylglycerol was measured with a BAS-2500 imaging system (Fuji Film, Tokyo, Japan). The inhibitory concentration (IC_{50}) of Compound-A with a 95% confidence interval (95% CI) was calculated using GraphPad Prism version 5.02 (GraphPad Inc., CA, USA).

2.3. Animals

Animal care and all animal experimental procedures were conducted according to the guidelines approved by the Animal Experimental Use Committee of the Takeda Pharmaceutical Company, and every effort was made to minimize the number and any suffering of the animals used in the experiments.

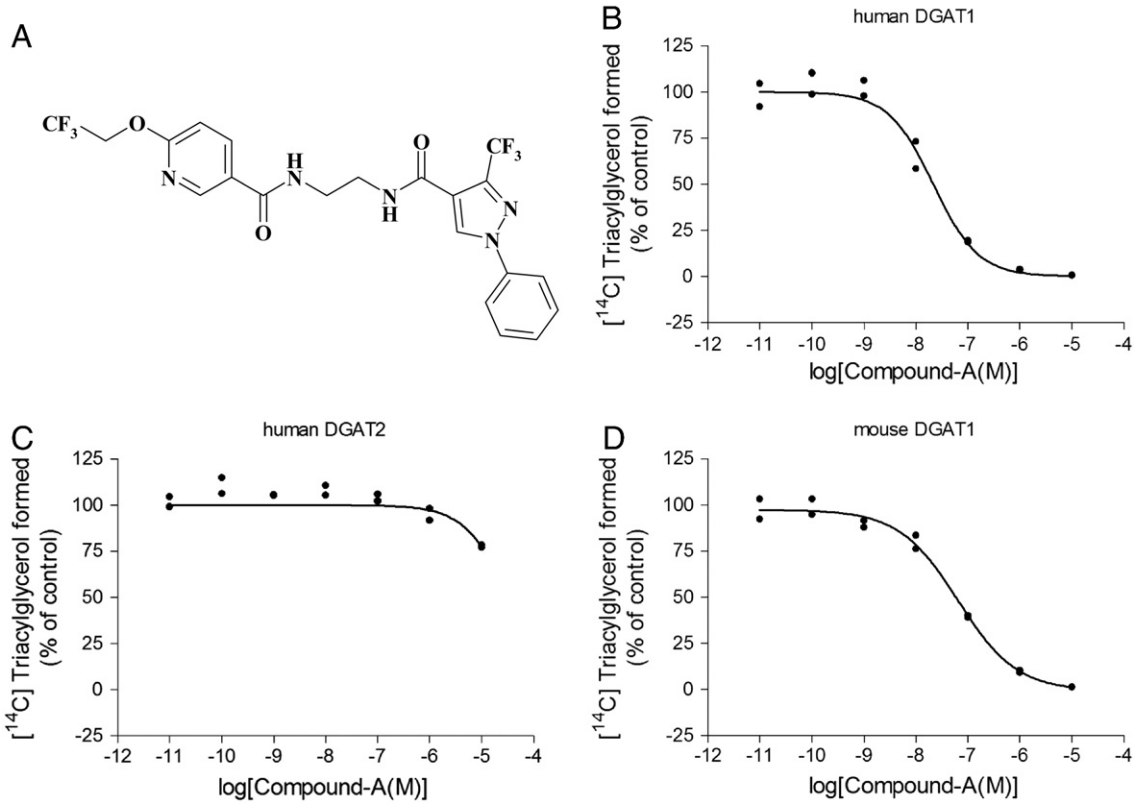

Fig. 1. Chemical structure and DGAT enzyme inhibitory activities of Compound-A. Chemical structure of Compound-A are shown (A). The dose–response curves of Compound-A against the recombinant hDGAT1 (B), hDGAT2 (C), and mDGAT1 (D) are shown with 95% CI (the upper and lower limits shown by circles). The IC_{50} of Compound-A against recombinant hDGAT1 and mDGAT1 were 22 nM (95% CI: 15–30 nM) and 64 nM (95% CI: 42–98 nM), respectively, and >10,000 nM against hDGAT2.