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JTP-103237, a novel monoacylglycerol acyltransferase inhibitor, modulates fat absorption and prevents diet-induced obesity

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ABSTRACT

Monoacylglycerol acyltransferase 2 (MGAT2) plays an important role in intestinal fat absorption. We discovered the novel MGAT2 inhibitor, JTP-103237, and evaluated its pharmacological profile. JTP-103237 selectively inhibited MGAT2 without remarkable species differences and reduced absorbed lipids in circulation. After lipid administration, JTP-103237 slightly but significantly decreased triglyceride content in proximal small intestine and significantly increased the lipids content in the distal small intestine. In addition, JTP-103237 significantly increased MGAT substrate (monoacylglycerol and fatty acid) content in the small intestine. JTP-103237 increased plasma peptide YY levels after lipid loading and reduced food intake in a dietary fat-dependent manner. After chronic treatment, JTP-103237 significantly decreased body weight and increased O_2 consumption in the early dark phase in high fat diet induced obese (DIO) mice. Moreover, JTP-103237 improved glucose tolerance and decreased fat weight and hepatic triglyceride content in DIO mice. Our findings indicate that JTP-103237 prevents diet-induced obesity by inhibiting intestinal MGAT2 and has unique properties as a drug for the treatment of obesity.

1. Introduction

The prevalence of obesity is increasing around the world and effective treatment strategies are urgently needed to address the obesity epidemic (Halford et al., 2010; Rodgers et al., 2012). Obesity results from an imbalance between energy intake and energy expenditure (Hill et al., 2012). Understandably, an energy imbalance is caused by excessive dietary fat intake (Prentice and Poppitt, 1996) which has been implicated as promoting not only obesity, but also metabolic diseases such as type 2 diabetes (Bray and Popkin, 1998; van Dam et al., 2002).

Monoacyglycerol acyltransferases (MGATs) catalyze in the first step of triglyceride (TG) synthesis and are involved in dietary fat absorption. The formation of diacylglycerol (DG) from monoacylglycerol (MG) and fatty acyl CoA by MGAT is considered the rate-limiting step of triacylglycerol synthesis in intestine (Senior and Isselbacher, 1962; Yen and Farese, 2003). The glycerol 3-phosphate pathway, which is another pathway for TG synthesis, is mainly involved in the de novo TG synthesis in most tissues (Lehner and Kuksis, 1996). Three subtypes of MGAT have been identified. Among these, MGAT2 is highly expressed in the intestine and is expressed in both humans and rodents (Yen and Farese, 2003). Thus, it is likely that MGAT2 plays an important role in dietary fat absorption. In fact, the absorption of fat into circulation was significantly reduced in MGAT2 knockout (KO) mice (Tsuchida et al., 2012). In addition, diet induced obesity, glucose intolerance and fatty livers were prevented in MGAT2 deficient mice (Yen et al., 2009). Moreover, MGAT2 KO mice demonstrated increased energy expenditure as compared to wild-type mice and the differences were most pronounced during the feeding period, suggesting that MGAT2 modulates diet-induced thermogenesis (Yen et al., 2009; Nelson et al., 2011). Thus, there is a strong possibility that inhibiting MGAT2 is a promising strategy for metabolic disorders caused by excessive fat intake, such as obesity and type 2 diabetes.

Although the physiological role of MGAT2 has been actively researched using MGAT2 KO mice, there have been no reports about the detailed pharmacological profile of MGAT2 inhibitors. Recently, we discovered the novel MGAT2 inhibitor, JTP-103237, which is a 7-(4,6-Di-tert-butyl-pyrimidin-2-yl)-3-(4-trifluoromethoxy-phenyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-a]

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pyrazine derivative. In this study, we evaluated the effects of JTP-103237 on lipid metabolism in the intestine, energy expenditure and diet-induced obesity.

2. Materials and methods

2.1. Materials

JTP-103237 was synthesized in the Central Pharmaceutical Research Institute of Japan Tobacco Inc. (Osaka, Japan). [1-^{14}C] oleoyl-coenzyme A (oleoyl-CoA) was purchased from Amersham Biosciences. [carboxyl-^{14}C] triolein was purchased from PerkinElmer. All other chemicals were of standard reagent grade.

2.2. Animals and diets

All animals were purchased from Charles River Laboratories (Yokohama, Japan). The animals were maintained on CRF-1 (Charles River Japan), as standard laboratory chow diets, and water ad libitum. For the evaluation of food consumption and for repeated administration studies, animals were given free access to water and experimental diets. The diets contained 3.1% and 35% (w/w) fat were purchased from Oriental Yeast Co. (Osaka, Japan). The animals were housed under specific pathogen-free conditions in a room controlled for temperature at 23 ± 3 °C and humidity of 55 ± 15% in 12-h light/dark cycles (lights on from 8:00 a.m. to 8:00 p.m.). All procedures were conducted according to guidelines from Japan Tobacco's Animal Care Committee.

2.3. Enzyme assays

Recombinant human MGAT2 (hMGAT2) or MGAT3 (hMGAT3) were cloned into pcDNA3.1 (+) vectors and transiently transfected into COS-7 cells using lipofectamine 2000. Membrane fractions of COS-7 cells were isolated as enzyme sources.

Recombinant human diacylglycerol acyltransferase (DGAT) 2 (hDGAT2) was cloned into pFASTBAC1 vectors and expressed in Sf9 insect cells using a baculovirus expression system. Sf9 cells were infected and membrane fractions isolated as enzyme sources as described by Cases et al. (1998). The reaction mixtures for hMGAT2 and hMGAT3 enzyme assays contained 100 mM Tris–HCl (pH 7.5), 250 mM Sucrose, 5 mM MgCl2, 0.05% bovine serum albumin (BSA), 0.05 mM 2-oleoyl glycerol and 1 μg of protein/ml of recombinant hMGAT2 or 15 μg of protein/ml of recombinant hMGAT3. The reaction mixtures for the hDGAT2 enzyme assay contained 100 mM Tris–HCl (pH 7.5), 250 mM Sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 0.1% BSA, 0.2 mM 1,2-oleoyl-sn-glycerol and 30 μg of protein/ml of recombinant hDGAT2. Serial dilutions of JTP-103237 with DMSO were added to the reaction mixture at a final concentration of 5% DMSO. The reaction was initiated by adding 25 μM of [1-^{14}C] oleoyl-CoA for a final volume of 100 μl and the mixture was incubated for 15 min at 25 °C. The reaction was terminated by adding 500 μl chloroform: methanol (2:1) solvent. After mixing, the reaction mixture was centrifuged (2000*g*, 10 min) and lipids extracted in the organic phase were separated by thin layer chromatography (TLC) using a hexane:diethylether:acetic acid (80:30:2) solvent system. The radioactivity of synthesized [1-^{14}C] Diacylglycerol was measured using the FLA-7000 imaging system (Fuji film, Tokyo, Japan). Human, rat and mouse intestinal microsomal MGAT enzyme assays were performed in the same manner as the MGAT2 enzyme assay, except for the use of 15 μg/ml of human intestinal microsomes (KAC, Kyoto, Japan), 6 μg/ml rat intestinal microsomes or 4 μg/ml mouse intestinal microsomes instead of recombinant hMGAT2. IC50 values were calculated in a semilogarithmic proportional manner from the two points flanking 50% inhibition.

2.4. Evaluation of intestinal MGAT activity

These experiments were performed in order to select the dose in in vivo experiments. JTP-103237 (10, 30 and 60 mg/kg) or vehicle (0.5% methylcellulose) was administered orally to SD rats (7 weeks old). Small intestines were collected over time at 1 and 8 h after administration of JTP-103237 from individual rats. Collected portions of the intestine (almost 100 mg) were carefully washed and weighed. All of these samples were collected in the same portion of the small intestine. These samples were homogenized with 1 ml/100 mg tissue of homogenate buffer consisting of 0.25 M sucrose, 100 mM of Tris–HCl and a zirconia ball (YTZ® ball, Nikkato Corporation) using a mixer mill (Retsch Co., Ltd.). The homogenate was subjected to centrifugation at. 10,000*g* for 30 min at 4 °C and the resulting supernatant was designated the enzyme fraction (S9 fraction). MGAT activity in the enzyme fraction was measured using the same method described above (Section CNDJ6nn5us4RjIIAqgBLqQsCAAAACAAAAA4AAABfAFIAZQBmADQAMQAxADYAMgA5ADEANAA3AAAA 2.3).

In mice (8-week old male C57BL/6J mice), JTP-103237 (10, 30 and 100 mg/kg) or vehicle (0.5% methylcellulose) was administered orally and small intestines were collected 2 h after administration. The method for S9 perpetration and measurement of MGAT activity were also the same as the method mentioned above and the percent of the MGAT activity in the control group was calculated. Since the number of animals in these studies was 3 or less, statistical analysis was not performed.

2.5. Evaluation of fat absorption

12–14-week old male C57BL/6J mice were fasted before turning off the lights on the day prior to administration. JTP-103237 (100 mg/kg) or vehicle (0.5% methylcellulose) was administered orally to fasted mice. 30 min after administration, 54 μCi/kg of [1-^{14}C] triolein in a 2% lipid emulsion was orally gavaged. Immediately prior to this procedure, 10% of Triton WR-1339 was intravenously administered as 5 ml/kg body weight. Blood samples were collected 30 and 60 min after lipid loading from ophthalmic veins and radioactivity was measured with a scintillation counter.

2.6. Evaluation of distribution of lipid components in the intestine

The method of compound administration and radiolabeled lipid loading were the same as in the evaluation of fat absorption. Three segments of small intestine were individually and periodically collected 15, 30 and 60 min after lipid loading. Lipids were extracted from tissues, and were developed and separated via TLC. The signal intensity of TG, DG, fatty acids and MG fractions on a TLC plate was measured using a FLA-7000 imaging system as described above (Section CNDJ6nn5us4RjIIAqgBLqQsCAAAACAAAAA4AAABfAFIAZQBmADQAMQAxADYAMgA5ADEANAA3AAAA 2.3). The equivalent radioactivity in each sample was calculated using a correction factor obtained from the standard sample.

2.7. Glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) levels after lipid loading

7-week old male SD rats were fasted before turning off the lights on the day prior to administration. JTP-103237 (30 mg/kg) or vehicle (0.5% methylcellulose) was subsequently orally administered 30 min prior to the loading of the 10 ml/kg of lipid emulsion (Intralipos® Injection 20%, Otsuka Pharmaceutical Factory, Inc.,