Pharmacological Characterization of Diethyl-2-\{(3-dimethylcarbamoyl-4-\[(4'-trifluoromethyl)biphenyl-2-carbonyl)amino\]phenyl}acetyloxymethyl\}-2-phenylmalonate (JTT-130), an Intestine-Specific Inhibitor of Microsomal Triglyceride Transfer Protein

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Received August 6, 2010; accepted October 21, 2010

ABSTRACT

Inhibitors of microsomal triglyceride transfer protein (MTP) expressed in the liver and small intestine are potential candidates for lipid-lowering agents. However, inhibition of hepatic MTP could lead to significant safety issues such as fatty liver disease. To develop a specific inhibitor of intestinal MTP, JTT-130 [diethyl-2-\{(3-dimethylcarbamoyl-4-\[(4'-trifluoromethyl)biphenyl-2-carbonyl)amino\]phenyl}acetyloxymethyl\}-2-phenylmalonate], was designed to be rapidly hydrolyzed in the absorption process. Here, we describe JTT-130, an intestine-specific MTP inhibitor, and evaluate its pharmacological properties. In vitro metabolic stability tests, JTT-130 was readily hydrolyzed during incubation with liver S9 from humans, hamsters, and rats. In an in vitro triglyceride (TG) transfer assay with human intestinal MTP, JTT-130 potently inhibited TG transfer activity with an IC50 value of 0.83 nM. When orally administered to hamsters, JTT-130 significantly suppressed an increase in chylomicron-TG after olive oil loading at 0.3 mg/kg and above but did not inhibit TG secretion from the liver at doses of up to 1000 mg/kg, indicating an inhibitory action highly specific for the small intestine. In rats orally administered [14C]triolein, JTT-130 potently suppressed an increase in blood 14C radioactivity and increased 14C radioactivity in the upper small intestine and the intestinal lumen. In hyperlipidemic hamsters fed a high-fat and high-cholesterol diet, repeated dosing with JTT-130 for 2 weeks reduced TG and cholesterol levels in the plasma and TG content in the liver. These results indicated that JTT-130 is a potent inhibitor specific to intestinal MTP and suggested that JTT-130 would be a useful compound for the treatment of dyslipidemia without inducing hepatotoxicity.

Introduction

Metabolic syndrome is characterized by the coexistence of metabolic risk factors for coronary heart disease (CHD), such as obesity, insulin resistance, glucose intolerance, and dyslipidemia, in an individual (Zimmet et al., 2001). People with metabolic syndrome are exposed to increased risk of CHD (Nakamura et al., 2001). Metabolic syndrome is considered to be preventable by dietary manipulation (Riccardi and Rivellese, 2000). Western diets (high-fat and high-cholesterol diets) are well known to induce hyperlipidemia, obesity, and insulin resistance, all of which, in turn, may increase the risk for CHD. Microsomal triglyceride transfer protein (MTP) in enterocytes, which plays an important role in intestinal lipids absorption, is one of the targets for drug discovery in the field of metabolic diseases.

MTP, localized in the endoplasmic reticulum in hepatocytes and enterocytes, transfers triglyceride (TG) and cholesteryl ester (CE) between membranes (Gordon et al., 1995; Wetterau et al., 1997; Olofsson et al., 1999). It participates in the assembly of TG-rich lipoproteins, such as chylomicron particles in the small intestine and very low-density lipoprotein (VLDL) particles in the liver. Since the in vivo effects of MTP inhibitors were reported (Wetterau et al., 1998), it has been pointed out that inhibition of hepatic MTP could lead to

ABBREVIATIONS: CHD, coronary heart disease; MTP, microsomal triglyceride transfer protein; TG, triglyceride; CE, cholesteryl ester; VLDL, very low-density lipoprotein; BAY 13-9952, implitapide; BMS-201038, lomitapide mesylate; SD, Sprague-Dawley; PC, phosphatidylcholine; KO, knockout; TGSR, TG secretion rate; JTT-130, diethyl-2-\{(3-dimethylcarbamoyl-4-\[(4'-trifluoromethyl)biphenyl-2-carbonyl)amino\]phenyl\}acetyloxymethyl\}-2-phenylmalonate; Apo, apolipoprotein; SUV, small unilamellar vesicle; DEAE, diethylaminoethyl; HF/HC, high-fat and high-cholesterol.
the potent blockade of VLDL release, resulting in reduced plasma lipids but inducing fatty liver and hepatic dysfunction (Shiomi and Ito, 2001; Burnett and Watts, 2007). In fact, although the potential benefits of MTP inhibition, such as lowering chylomicron-TG and VLDL-TG levels, were demonstrated in animal experiments and in clinical studies, several major toxicity issues confronted the clinical development of MTP inhibitors (Shiomi and Ito, 2001; Chandler et al., 2003). In clinical studies of BAY 13-9952 (implitapide) and BMS-201038 (lorlatipide mesylate), for example, hepatotoxicity indicated by the elevation of transaminase level halted their developments. Therefore, we postulated that compounds designed to selectively inhibit intestine-MTP would need to be novel lipid-absorption inhibitors without any hepatotoxicity. In our strategy to obtain compounds, which might show little tendency to inhibit hepatic MTP after oral administration, we designed compounds that would be rapidly metabolized during the absorption process. In the studies described herein, we demonstrate the specificity of JTT-130 to intestinal MTP and also describe its effect on lipid metabolism to evaluate the pharmacological potential of JTT-130 as an intestine-specific MTP inhibitor.

Materials and Methods

Compounds. JTT-130 and its major metabolite (M1) were synthesized at the Central Pharmaceutical Research Institute, Japan Tobacco Inc. (Osaka, Japan). [14C]JTT-130 was synthesized at Sekisui Medical Co., Ltd. (formerly known as Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan).

Animals. Male Syrian hamsters and male Sprague-Dawley (SD) rats were purchased from Japan SLC (Shizuoka, Japan) and Charles River Japan (Yokohama, Japan), respectively. The animals were maintained on CE-2 (Oriental Yeast, Tokyo, Japan) or CRF-1 (Charles River Japan) as standard laboratory chow diets and water ad libitum. The animals were housed under specific pathogen-free conditions in a room controlled for temperature at 23°C and humidity of 55 ± 15% in 12-h light/dark cycles (lights on from 8:00 AM to 8:00 PM). These studies complied with the Guidelines for Animal Experimentation at our laboratories.

Metabolic Stability In Vitro. Liver supernatant fraction, derived by centrifugation at 9000g (liver S9) from human, hamsters, and rats, was purchased from XenoTech LLC (Lenexa, KS). [14C]JTT-130 was added to plasma or liver S9 (2 mg/ml protein) from human, hamsters, and rats at a final concentration of 5 µg/ml, and the mixtures were incubated at 37°C. After incubation, the reaction mixtures were deproteinized with acetonitrile, and the 14C radioactivity in the obtained supernatant fractions was analyzed by radio-label high-performance liquid chromatography detection for the detection of JTT-130 and its metabolites.

Triglyceride and Cholesteryl Ester Transfer Activities by MTP. TG and CE transfer activities of MTP were assayed by the method of Wetterau et al. (1992) with small modifications. [Carboxyl-14C]triolein ([14C]TG) (3.7 MBq/ml), [cholesteryl-4-14C]cholesteryl oleate ([14C]CE) (3.7 MBq/ml), and [2-palmitoyl-9-10-3H(N)]phosphatidylcholine ([3H]PC) (3.7 MBq/ml) were purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). In brief, donor small unilamellar vesicles (SUVs) were prepared by bath sonication of a mixture containing 13 µmol of phosphatidylcholine, 650 nmol of cardiolipin, and 32.5 nmol of unlabeled TG or CE, and 200 kBq [14C]TG or [14C]CE. Acceptor SUVs were composed of 26 µmol of phosphatidylcholine, 65 nmol of unlabeled TG or CE, and 20 kBq of [3H]PC. Each mixture was centrifuged at 159,000g for 2 h at 4°C to collect the supernatants containing SUVs. MTP, partially purified from microsomes of small intestine (human) and liver (humans, hamsters, and rats) (Tissue Transformation Technologies, Edison, NJ or XenoTech LLC) using a fast protein liquid chromatograph (GE Healthcare, Little Chalfont, Buckinghamshire, UK) equipped with a DEAE-Sepharose column, was added to a reaction mixture (final reaction volume; 500 µl) containing donor SUVs, acceptor SUVs, and JTT-130 (final concentrations; 0.01-30 nM). After incubation at 37°C, the reaction was terminated by the addition of 1250 µl of a 33% (w/v) DEA-cellulose suspension. After agitation, the donor SUVs, bound to DEA-cellulose, was selectively sedimented by centrifugation at 15,000g for 3 min. The 14C radioactivity in the supernatant containing the acceptor SUVs was measured to assess the lipids transferred from the donor SUVs. 3H radioactivity, a marker of acceptor SUVs, was also measured to correct for the lipid transfer activity.

Apolipoprotein Secretion from Caco-2 Cells. Caco-2 cells (American Type Culture Collection, Manassas, VA), human intestinal epithelial cell line, were cultured in Eagle’s minimum essential medium containing 10% heat inactivated fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin and 100 µM nonessential amino acids at 37°C in a humidified, 5% CO2 atmosphere. For the experiments, the cells were plated on 24-mm diameter Transwell filters at a density of 3.6 × 10^5 cells/filter and grown for approximately 16 days. The cells were incubated with 0.8 mM oleate and various concentrations of JTT-130 (final concentration; 0.1-100 nM) for approximately 20 h, and the culture medium obtained from the basolateral side was used for the measurement of apolipoprotein (Apo) concentration. ApoB concentrations in the culture media were measured by sandwich enzyme-linked immunosorbent assay using monoclonal anti-human apolipoprotein B (Seradyn, Indianapolis, IN) and sheep anti-human apolipoprotein B-peroxidase conjugate (The Binding Site, Birmingham, UK), as described by Haghparast et al. (1996). The ApoA1 concentrations in the culture media were measured by enzyme-linked immunosorbent assay (Mabtech, Nacka Strand, Sweden).

Triglyceride Absorption in Hamsters. To evaluate inhibitory effects on fat absorption, nonfasted Syrian hamsters (male, 9 weeks old) were orally administered JTT-130 suspended in 0.5% (w/v) methyl cellulose solution. At 30 min after dosing, olive oil (2 ml/kg body weight; Wako Pure Chemical Industries, Osaka, Japan) was loaded orally. Blood samples were taken from the orbital venous plexus under ether anesthesia before and 4 h after olive oil loading to measure serum TG concentrations. Lipoproteins in the serum were separated by agarose gel electrophoresis (Titan gel lipoprotein; Helena Laboratories, Saitama, Japan), and TG was stained with Titan stain. Lipoproteins were separated by agarose gel electrophoresis (Titan gel lipoprotein; Helena Laboratory, Saitama, Japan), and TG was stained with Titan stain. The density in each of the lipoprotein fractions was analyzed using a densitometer at 570 nm (CS-9300PC; Shimadzu, Kyoto, Japan) to determine the ratio of chylomicron-TG to total serum TG level. The concentrations of chylomicron-TG in the serum were calculated from the serum TG concentrations and the fraction ratio of chylomicron-TG.

Hepatic Triglyceride Secretion in Hamsters. The TG secretion rate (TGSR) in hamsters was assessed according to a method described previously (Sasase et al., 2007). Overnight fasted hamsters (male, 9 weeks old) were orally administered JTT-130 (100, 300, and 1000 mg/kg) suspended in 0.5% (w/v) methyl cellulose solution, and 30 min after dosing, Triton WR-1339 (20% w/v in saline, 400 mg/kg body weight; Sigma-Aldrich, St. Louis, MO) was injected intravenously. Blood samples were collected at 0, 30, 60, and 120 min after Triton WR-1339 injection. The TGSR was determined from the rate of increase in the serum TG level per minute.

Triglyceride Metabolism in Rats. Nonfasted SD rats (male, 10 weeks old) were orally administered JTT-130 at a dose of 10 mg/kg dissolved in polyethylene glycol 400. Each animal was dissected, and a large portion of the intestine was removed (from the pylorus to the cecum) at 2 h after dosing. The obtained portion was incised, fixed with 10% formalin solution, and stained with Sudan IV to detect lipid deposition in the epithelial layer. Nonfasted male 10-week-old SD rats were orally administered JTT-130 at a dose of 10 mg/kg. Thirty minutes later, olive oil (100 µl/head) containing [14C]triolein (185 kBq) and [3H]sitostanol (185 kBq)
Fluor was added to the samples, the radioactivity of $^{14}$C and $^3$H was administered twice a day, and fecal samples were collected to measure in the upper intestinal tissue and the luminal contents in the small intestine and cecum. The recovery of $^{14}$C and $^3$H radioactivity was expressed as a percentage of the total $^{14}$C and $^3$H radioactivity loaded, and the $^{14}$C/$^3$H ratio in the contents was calculated.

Cholesterol Metabolism in Hamsters. Nonfasted hamsters (male, 10 weeks old) were administered JTT-130 orally (0.3-30 mg/kg) at 30 min before oral administration of olive oil (2 ml/kg body weight) containing $[^{14}$C]cholesterol (370 kBq/kg). Blood samples were obtained at 4 h after radiolabeled cholesterol loading, and the $^{14}$C radioactivity in blood was measured using a liquid scintillation counter.

JTT-130 was administered orally at doses of 1, 3, and 10 mg/kg to nonfasted hamsters (male, 10 weeks old) 30 min before administration of olive oil (200 μ/lhead) containing $[^{14}$C]cholesterol (34.7 kBq) and $[^3$H]sitostanol (72.4 kBq). For the following 72 h, JTT-130 was administered twice a day, and fecal samples were collected to measure the radioactivity of $^{14}$C and $^3$H. The collected feces were homogenized, decolored, and then dissolved in Solusene-350. After Hionic-Fluor was added to the samples, the radioactivity of $^{14}$C and $^3$H was measured with a liquid scintillation counter. The $^{14}$C/$^3$H ratio was calculated from the recovery of $^{14}$C and $^3$H radioactivity as described above.

Administration of JTT-130 in Hyperlipidemic Hamsters Fed a High-Fat and High-Cholesterol Diet. Male 9-week-old Syrian hamsters were acclimatized to a high-fat and high-cholesterol diet, which was limited to 7 g per day to supply the same amount of fat and cholesterol as the HF/HC diet, which was limited to 7 g/day (0.0016, or 0.0164% (w/w), respectively] for 14 days. The normal hamsters (male, 10 weeks old) were administered JTT-130 orally (0.3-30 mg/kg) at a dose equivalent to 1, 3, or 10 mg/kg/day [0.0016, or 0.0164% (w/w), respectively] for 14 days. The normal group was fed normal chow diet (CE-2, 7 g/day). Blood samples were collected from the orbital venous plexus under ether anesthesia on day 15, and the serum levels of total cholesterol and TG were measured. TG was extracted from the liver with chloroform-methanol (2:1) for determination of the hepatic TG content.

Statistical Analysis for In Vivo Studies. Data are presented as the mean ± S.E. Statistical analysis was performed between the control and other groups using the t test or Dunnett’s test (StatLight 1998; Yukms Corp., Tokyo, Japan). $p < 0.05$ was considered statistically significant.

Results

Metabolic Stability of JTT-130 In Vitro. JTT-130 was designed to be rapidly hydrolyzed to its inactive metabolite (M1) by cleavage of ester group in the structure (Fig. 1). The results of the in vitro stability of JTT-130 during incubation with plasma or liver S9 from human, hamsters, and rats are shown in Table 1. JTT-130 was completely hydrolyzed after 1-h incubation in rat plasma into M1. In human and hamster plasma, the residual ratios after 1-h incubation were 98.2 and 48.4%, respectively.

Effect of JTT-130 on Lipid Transfer by MTP and Apolipoprotein Secretion from Caco-2 Cells. MTP from human small intestine or liver can transfer radioactive TG and CE between the SUVs. JTT-130 potently inhibited lipid transfer by MTP derived from human small intestine in a concentration-dependent manner. The IC$_{50}$ values for TG and CE transfer were 0.83 and 0.74 nM, respectively (Table 2). M1, the major metabolite of JTT-130, is generated by hydrolysis of the ester bond in JTT-130 (Fig. 1). No inhibitory effect of M1 on MTP was observed at concentrations of M1 increasing up to 30,000 nM. To evaluate the species differences in lipid transfer activity, the inhibitory effects of JTT-130 on TG transfer activity by MTP from other species, including rats and hamsters, were also investigated. JTT-130 inhibited TG transfer by hepatic MTPs derived from human, hamsters, and rats with the IC$_{50}$ values of 0.25 ± 0.03, 0.51 ± 0.09, and 0.21 ± 0.05 nM,

![Fig. 1. Chemical structure of JTT-130 and its metabolite (M1). JTT-130 was designed to be rapidly hydrolyzed to inactive metabolite (M1) after absorption from the small intestine. * represents the $^{14}$C radiolabeling position in the JTT-130 used in the in vitro metabolic stability tests.](image-url)
Inhibitory effects of JTT-130 and its metabolite (M1) on the secretion of ApoB and ApoA1 from Caco-2 cells

To assess a selective inhibitory effect of JTT-130 on ApoB-containing lipoprotein secretion, the ApoB and ApoA1 secreted from Caco-2 cells were measured in the presence of JTT-130 or M1. The IC$_{50}$ value was calculated in a semilogarithmic proportional manner from the two points enclosing 50% inhibition. Each value represents the mean ± S.E. from four independent experiments.

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<tr>
<th>Compound</th>
<th>ApoB IC$_{50}$</th>
<th>ApoA1 IC$_{50}$</th>
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<tr>
<td>JTT-130</td>
<td>9.5 ± 3.1</td>
<td>&gt;30,000</td>
</tr>
<tr>
<td>M1</td>
<td>&gt;30,000</td>
<td>&gt;30,000</td>
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respectively. There were no species differences in the potency of inhibition, the IC$_{50}$ values for these species being comparable with that for human. The M1 showed no inhibitory effect on MTPs derived from each species up to 30,000 nM.

MTP participates in secretion of ApoB-containing lipoprotein by assembling lipid and ApoB. To assess a selective inhibitory effect of JTT-130 on ApoB containing lipoprotein secretion, the ApoB and ApoA1 secreted from Caco-2 cells were measured in the presence of JTT-130. JTT-130 inhibited ApoB secretion with an IC$_{50}$ value of 9.5 nM but did not affect ApoA1 secretion (Table 3). On the other hand, M1 affected neither ApoB secretion nor ApoA1 secretion at concentrations up to 30,000 nM.

**Specific Inhibition of JTT-130 to Intestinal MTP in Hamsters.** To assess whether the in vitro instability of JTT-130 reflects on its in vivo metabolism, TG absorption from small intestine and TG secretion from liver were investigated in hamsters receiving JTT-130 orally. The inhibitory effect of JTT-130 on intestinal fat absorption was investigated based on the plasma chylomicron-TG and TG levels after olive oil loading. JTT-130 significantly suppressed increases in chylomicron-TG levels after olive oil loading at 0.3 mg/kg and above (Fig. 2A) as well as increases in plasma TG levels at the dose of 1 mg/kg and above (Fig. 2B). The efficacies of JTT-130 on plasma TG suppression were 30.7, 57.1, and 65.5% at 1, 3, and 10 mg/kg, respectively.

The effect of JTT-130 on liver MTP was evaluated based on TGSR from liver in Triton WR-1339-treated hamsters. JTT-130 showed no inhibitory effect on TG secretion from the liver after Triton WR-1339 injection at doses up to 1000 mg/kg (Fig. 2C). These results clearly showed a marked difference in the action of JTT-130 between the liver and the intestine. The specific inhibition of JTT-130 to intestinal MTP was also supported by pharmacokinetic data in hamsters, in which JTT-130 was not detected in plasma at all but hydrolyzed metabolite was detected ($C_{max}$ 7.3 ng/ml at 2 h after dosing) as expected when JTT-130 (40 mg/kg) was orally administered to fasted hamsters (M. Suzuki, unpublished data).

**Effect of JTT-130 on Triglyceride Metabolism in Rats.** To examine the pharmacological properties of JTT-130, we assessed the effects on TG and cholesterol metabolism in rats and hamsters. First, intestinal TG metabolism was investigated in rats treated with JTT-130 (10 mg/kg p.o.). The fat in small intestine was stained with Sudan IV at 2 h after dosing JTT-130. Intestinal specimens from the JTT-130 group were stained intensely, particularly around the duodenum and jejunum, whereas those from the vehicle group were not stained (Fig. 3A).

$[^{14}C]$Triolein and $[^{3}H]$sitostanol (unabsorbable sterol used as an internal standard) were administered orally to rats pretreated orally with JTT-130 (10 mg/kg). At 4 h after radiolabelled TG loading, JTT-130 increased recovery of $^{14}$C radioactivity, a percentage of the total $^{14}$C radioactivity loaded, in the upper intestinal tissue (Fig. 3B) and suppressed increase in $^{14}$C radioactivity in the blood (Fig. 3C). JTT-130 increased the $^{14}$C/$^{3}$H ratio in the luminal contents in the upper small intestine as well as in the lower small intestine and cecum (Fig. 3D).

**Effect of JTT-130 on Cholesterol Metabolism in Hamsters.** The effect of JTT-130 on cholesterol metabolism was investigated in hamsters treated with JTT-130. The effect on cholesterol absorption and excretion were evaluated in hamsters loaded with $[^{14}C]$cholesterol and $[^{3}H]$sitostanol. JTT-130 (0.3-30 mg/kg), administrated orally before dosing with $[^{14}C]$cholesterol, significantly decreased the $^{14}$C radioactivity in blood obtained 4 h after loading at 3 mg/kg and above (Fig. 4). To measure the cholesterol excretion in the feces, we administrated JTT-130 (1, 3, and 10 mg/kg p.o. b.i.d.) to hamsters and collected the feces for 3 days. The recovery of $^{14}$C radioactivity in the feces, a percentage of the total $^{14}$C radioactivity in the feces, was 30.7, 57.1, and 65.5% at 1, 3, and 10 mg/kg, respectively.

![Fig. 2](https://example.com/fig2.png)

**Fig. 2.** Selective inhibition of intestinal MTP in hamsters. JTT-130 was administered orally to hamsters before 30 min before the olive oil loading. A and B, the chylomicron-TG and TG levels in plasma were measured before and 4 h after olive oil loading, and the change in the chylomicron-TG level (A) and the change in plasma TG level (B) were calculated. Each value represents the mean ± S.E. (n = 8/group). *** p < 0.001; * p < 0.05 versus control (Dunnett's test). To evaluate the effect of JTT-130 on hepatic-MTP, TG secretion from the liver was measured in Triton WR-1339-treated rats. C, TGSR is shown. Each value represents the mean ± S.E. (n = 5–6/group).
radioactivity loaded, showed a slight dose-dependent increase in the JTT-130-treated groups. On the other hand, fecal recovery of $^3$H radioactivity from $[^3]$Hsitostanol used as the internal standard did not differ between the groups. The fecal $^{14}$C/$^3$H ratio tended to increase in the JTT-130-treated groups (Table 4).

**Effects of 2-Week Administration of JTT-130 on Lipid Metabolism in Hyperlipidemic Hamsters Fed a High-Fat and High-Cholesterol Diet.** The effects of 2-week administration of JTT-130 as dietary admixture on the serum levels and liver fat contents were investigated using hyperlipidemic hamsters fed a HF/HC diet. The actual mean dosages were calculated as 1.0, 3.1, and 10.6 mg/kg/day. JTT-130 reduced serum total cholesterol levels by 12 mean dosages were calculated as 1.0, 3.1, and 10.6 mg/kg/day (Fig. 3). The actual serum levels and liver fat contents were investigated under the fat-absorbing region, and the cecum were removed, and the luminal contents in each site were collected. B, the $^{14}$C radioactivity was measured in the upper intestinal tissue, and the recovery of $^{14}$C radioactivity was expressed as a percentage of the total $^{14}$C radioactivity loaded. C, the $^{14}$C radioactivity in blood was measured at 2 and 4 h after radiolabeled TG loading. D, the $^{14}$C and $^3$H radioactivity in the luminal were measured, and the $^{14}$C/$^3$H ratio was calculated from the recovery of $^{14}$C and $^3$H radioactivity. Each value represents the mean ± S.E. (n = 6/group). **, p < 0.01; *, p < 0.05 versus control (t test).

**Fig. 3.** Effect on triglyceride metabolism in rats. The small intestine from the rats was stained with Sudan IV at 2 h after JTT-130 administration (10 mg/kg). A representative example of stained small intestine is shown in A. To evaluate the effect of JTT-130 on TG metabolism, JTT-130 was administered orally to rats at a dosage of 10 mg/kg before olive oil containing $[^{14}]$triolein and $[^3]$Hsitostanol. The small intestine was removed and divided in the middle into two equal segments at 4 h after JTT-130 administration. The upper small intestine (stomach side; the fat-absorbing region), the lower small intestine (cecum side; segment beyond the fat-absorbing region), and the cecum were removed, and the luminal contents in each site were collected. B, the $^{14}$C radioactivity was measured in the upper intestinal tissue, and the recovery of $^{14}$C radioactivity was expressed as a percentage of the total $^{14}$C radioactivity loaded. C, the $^{14}$C radioactivity in blood was measured at 2 and 4 h after radiolabeled TG loading. D, the $^{14}$C and $^3$H radioactivity in the luminal were measured, and the $^{14}$C/$^3$H ratio was calculated from the recovery of $^{14}$C and $^3$H radioactivity. Each value represents the mean ± S.E. (n = 6/group). **, p < 0.01; *, p < 0.05 versus control (t test).

**Table 4** Effects of JTT-130 on cholesterol excretion in hamsters

The effects of JTT-130 on cholesterol excretion were evaluated in hamsters loaded with $[^{14}]$Ccholesterol and $[^3]$Hsitostanol. Fecal sample were collected for 3 days after initial administration of JTT-130, and fecal cholesterol was extracted as described under Materials and Methods. The radioactivity of $^{14}$C and $^3$H in the feces was measured. The recovery of $^{14}$C and $^3$H radioactivity in the feces was expressed as a percentage of the total $^{14}$C and $^3$H radioactivity loaded, and the $^{14}$C/$^3$H ratio was calculated. Each value represents the mean ± S.E. (n = 6/group).

<table>
<thead>
<tr>
<th>Group</th>
<th>Fecal Recovery $^{14}$C %</th>
<th>$^3$H %</th>
<th>Fecal $^{14}$C/$^3$H Ratio</th>
<th>p Value</th>
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<tr>
<td>Control</td>
<td>31.74 ± 3.59</td>
<td>79.83 ± 0.91</td>
<td>0.19 ± 0.02</td>
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<tr>
<td>JTT-130 (1 mg/kg)</td>
<td>30.90 ± 4.96</td>
<td>79.14 ± 1.27</td>
<td>0.19 ± 0.03</td>
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<tr>
<td>JTT-130 (3 mg/kg)</td>
<td>41.07 ± 5.82</td>
<td>80.39 ± 0.56</td>
<td>0.25 ± 0.02</td>
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<tr>
<td>JTT-130 (10 mg/kg)</td>
<td>46.78 ± 5.38</td>
<td>80.35 ± 1.49</td>
<td>0.28 ± 0.04</td>
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(Fig. 5, A and B). JTT-130 led to decreases in the hepatic TG contents, with the decrement at 30 (3.1 mg/kg/day) and 43% (10.6 mg/kg/day), respectively (Fig. 5C).

**Discussion**

Excessive intake of Western diets is well known to increase the risk for CHD through induction of obesity and hyperlipidemia (Connor et al., 1986; Kratz, 2005). MTP inhibitors are one of the potential candidates for lipid-lowering agents by inhibiting dietary lipid absorption from the intestine. In the present study, we characterize the lipid-lowering action of JTT-130, which as an intestine-specific MTP inhibitor markedly inhibited absorption of both TG and cholesterol without exerting a direct inhibition on hepatic lipid metabolism when administered orally.

MTP is expressed in the small intestine and liver and plays an important role in chylomicron and VLDL synthesis through the assembly of lipids into TG-rich lipoproteins (Gor-
don et al., 1995; Wetterau et al., 1997; Olofsson et al., 1999). Several nonclinical and clinical studies have shown that inhibition of liver MTP attenuated hepatic VLDL output, which resulted not only in reduction of the plasma lipids but also in hepatic lipid accumulation and consequently hepatotoxicity (Shiomi and Ito, 2001; Wierzbicki et al., 2009). Therefore, an intestine-specific MTP inhibitor is preferable to avoid hepatotoxicity. Dirlotapide, as this class of drug, has been marketed for the treatment of obesity in dogs (Wren et al., 2007a, b). The compounds, a series based on dirlotapide, were developed for the treatment of obesity in dogs (Wren et al., 2007a, b). The compounds, a series based on dirlotapide, were designed to be easily metabolized after being absorbed from the intestine but transient increases in concentration of these compounds in the plasma after administration could not be excluded completely (Vu et al., 2009). Therefore, there was still a concern of hepatotoxicity with these compounds. JTT-130, an intestine-specific MTP inhibitor, was designed to be rapidly hydrolyzed and inactivated by cleavage of ester group in the structure immediately after intestinal absorption.

In the in vitro studies, JTT-130 strongly inhibited the MTP-mediated transfer of TG and CE as well as ApoB secretion from Caco-2 cells. On the other hand, a major metabolite of JTT-130, a product of hydrolysis, did not show any inhibition at concentrations of up to 30,000 nM, indicating that the inhibitory activity was attributed exclusively to the total structure of JTT-130. In fact, JTT-130 was easily hydrolyzed when incubated with liver S9 from different species, such as humans, hamsters, and rats. In the in vitro metabolic stability test, species difference was observed in plasma. JTT-130 was extremely less stable in rat plasma, with little of parent drug detected after 5 min of incubation (data not shown). On the other hand, JTT-130 was more stable in plasma from humans and hamsters than in plasma from rats. However, with liver S9, residual ratios of JTT-130 after 5 min of incubation were below 30% in all species. These results suggested that JTT-130 is likely to be rapidly hydrolyzed in the liver after absorption in these species.

The results of the in vitro metabolic stability tests were reflected in the in vivo tissue-selective effects of JTT-130; orally administered JTT-130 inhibited TG absorption in the small intestine but did not inhibit TG secretion from the liver, even at 1000 mg/kg in hamsters. In addition, in an ex vivo study with the intestine and liver from JTT-130-administered hamsters to show direct inhibition of the tissue MTP activities, MTP in the small intestine was inhibited but not MTP in the liver (data not shown). After JTT-130 was administered to hamsters at 40 mg/kg, the unchanged compound was not detected in the plasma; however, a large amount of M1 was detected. These results indicate that the selectivity of JTT-130 for the intestinal MTP was ascribed to the pharmacokinetic properties of JTT-130 after absorption from the intestine. On the other hand, BMS-201038 and BAY 13-9952 (Chandler et al., 2003), a nonspecific MTP inhibitor, increased transaminase levels presumably associated with fatty liver due to inhibition of hepatic MTP in clinical studies. In contrast with the above compound, JTT-130 did not increase transaminase levels in a 3-month clinical trial with patients with dyslipidemia, indicating that JTT-130 exerted activity only on intestinal-MTP in humans (H. Kon, unpublished data).

In small intestine-specific MTP knockout (KO) mice, there were observed lipid deposition in small intestinal microvilli, decreased levels of plasma TG and cholesterol, and increased fecal excretion of lipids (Xie et al., 2006). In the present study, the inhibitory effects of JTT-130 on TG absorption were evidenced in rats and hamsters. Lipid staining with Sudan IV on the intestine from rats receiving oral JTT-130 revealed that lipid deposition was restricted to the upper part of the small intestine, especially the jejunum, which was strongly stained, whereas the lower part was poorly stained. The distribution of lipid staining was coincident with that of MTP in the intestine; MTP was highly localized in the upper part of the duodenum and jejunum (Swift et al., 2005). In addition, when 14C incorporation into the upper small intestine was determined after oral administration of [14C]TG to rats, 14C radioactivity in the intestinal tissue was significantly increased by JTT-130 administration. These results demonstrated that lipid accumulation in the enterocytes was due to decreased chylomicron synthesis/secretion by inhibi-

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**Fig. 5.** Effect of 2-week administration of JTT-130 on lipid metabolism in hyperlipidemic hamsters fed a high-fat and high-cholesterol diet. Hamsters were acclimatized to restricted feeding (7 g/day) of a HF/HC diet to supply the same amount of fat and cholesterol to all animals. JTT-130 was administered orally to the hamsters as a food admixture (7 g/day) for 2 weeks. Serum lipid parameters were measured on day 15 under nonfasted conditions. A and B, plasma total cholesterol (A) and TG (B) levels were measured. C, hepatic TG was extracted with chloroform-methanol (2:1, v/v), and the hepatic TG content was measured. The control group was fed a HF/HC diet (7 g/day) without JTT-130, and the normal group was fed a basal diet (Norm diet). Each value represents the mean ± S.E. (n = 6/group). †††, p < 0.001; ††, p < 0.01; †, p < 0.05 versus normal (t test); ††††, p < 0.001; **, p < 0.01 versus control (Dunn’s test).
tion of MTP as observed in KO mice (Xie et al., 2006). Fecal excretion of ingested fat or cholesterol was consequently increased by JTT-130 administration. When [14C]TG was given orally to rats, the 14C levels in the luminal content were markedly increased by JTT-130, suggesting that JTT-130 increased the excretion of 14C radioactivity. When [14C]cholesterol was given orally, JTT-130 suppressed the increase in 14C levels in the blood and increased the amount of 14C radioactivity in the feces. These studies demonstrated the potential of JTT-130 to decrease food efficiency through the inhibition of dietary lipid absorption.

Several MTP inhibitors, effective on hepatic lipid metabolism, have been shown to markedly reduce plasma lipid levels in various hyperlipidemic animal models. Their potent actions are considered to be related to the inhibition of VLDL secretion from the liver. JTT-130, administered to hamsters on a HF/HC diet for 2 weeks, was effective on dyslipidemia, decreasing plasma TG and cholesterol levels by approximately 30 and 40% at a dose of 10.6 mg/kg, respectively. Hepatic TG content was markedly decreased by JTT-130 treatment without the elevation of transaminase, suggesting one of the benefits of decreased dietary lipid absorption in addition to the absence of inhibition of hepatic MTP. Lipid-lowering effects of JTT-130 treatment were demonstrated in guinea pigs, which is one of the suitable animal models because of their similarities to humans in terms of lipid metabolism (Fernandez, 2001; West and Fernandez, 2004). In guinea pigs fed a hypercholesterolemic diet, JTT-130 decreased the levels of low-density lipoprotein cholesterol and TG in the plasma without inducing hepatic TG accumulation (Aggarwal et al., 2005). These results were similar to our results from hamsters, which is also used for evaluating lipid metabolism. Therefore, marked inhibition of fat absorption might lead to amelioration of hepatic steatosis. The efficacy of JTT-130 observed in hamsters clearly demonstrated that specific inhibition of intestinal MTP is a useful approach to the treatment of dyslipidemia free from hepatotoxicity.

In summary, our studies demonstrated that JTT-130 is an intestine-specific inhibitor with little risk of liver toxicity due to rapid degradation in the absorption process. The in vivo efficacy of JTT-130 observed in hamsters and rats after oral fat loading was consistent with the phenotype of small intestine-specific MTP KO mice (Xie et al., 2006). Furthermore, oral repeated administration of JTT-130 showed the intensive lipid-lowering effects in hyperlipidemic hamsters, indicating that JTT-130 may be useful in the treatment of dyslipidemia without causing hepatotoxicity.

Acknowledgments

We thank Dr. Tomohiko Sasase and Naoto Ogawa (Japan Tobacco Inc.) for their support regarding the experimental techniques in this series of studies.

Authorship Contributions

Participated in research design: Mera, Odani, Kawai, and Hata. Conducted experiments: Mera, Odani, Kawai, Hata, and Suzuki. Contributed new reagents or analytic tools: Hagiwara and Katushima.

Performed data analysis: Mera, Odani, and Hata.

Wrote or contributed to the writing of the manuscript: Mera, Hata, and Kakutani.

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JTT-130, a Novel Intestine-Specific MTP Inhibitor