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Effect of JTT-705 on cholesteryl ester transfer protein and plasma lipid levels in normolipidemic animals

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Abstract

This study evaluated JTT-705, *S*-[2-([[1-(2-ethylbutyl)cyclohexyl]carbonyl]amino)phenyl]2-methylpropanethioate, as a cholesteryl ester transfer protein (CETP) inhibitor in several animal species. In vitro, JTT-705 inhibited plasma CETP activities of humans, rabbits, hamsters, cynomolgus monkeys and marmosets with IC_{50} values of 5.5, 1.0, 11.7, 2.4 and 6.3 μM, respectively. The thiol form (JTP-25203) also inhibited those activities with IC_{50} values of 2.8, 0.44, 0.52, 1.3 and 1.1 μM, respectively. Following oral administration to normolipidemic animals (rabbits, hamsters and marmosets), JTT-705 reduced plasma CETP activity, increased high density lipoprotein cholesterol (HDL-cholesterol), and decreased the ratio of non-HDL-cholesterol to HDL-cholesterol (atherogenic index) in all species. In marmosets, JTT-705 increased slow α-migrating lipoprotein (apolipoprotein E-rich HDL) in agarose gel electrophoresis, indicating that HDL metabolism in JTT-705-treated marmosets is similar to that in CETP-deficient humans. These results indicate that JTT-705 can be expected to inhibit plasma CETP activity and improve plasma lipoprotein profiles in a wide range of animal species, including humans.

Keywords: JTT-705; Cholesteryl ester transfer protein (CETP) inhibitor; High density lipoprotein (HDL) cholesterol

1. Introduction

A high plasma level of low density lipoprotein cholesterol (LDL-cholesterol) is thought to be a risk factor for developing coronary heart disease (Castelli et al., 1986); however, a low plasma level of high density lipoprotein cholesterol (HDL-cholesterol) is also a risk factor (Corti et al., 1995; Barter and Rye, 1996). Cholesteryl ester transfer protein (CETP) is a plasma protein that transfers cholesteryl ester from HDL to LDL and very low density lipoprotein (VLDL), in association with a reciprocal transfer of triglyceride from LDL and VLDL to HDL (Lagrost, 1994). The importance of the role of CETP in the regulation of plasma HDL-cholesterol has gained support from studies of human genetic CETP deficiency (Inazu et al., 1996), studies in hamsters and rabbits in which CETP has been inhibited (Himanshu et al., 1997; Whitlock et al., 1989) and in a murine transgenic model (Agellon et al., 1991). Namely, a high CETP level may lead to a low HDL-cholesterol level and a low CETP level may induce a high HDL-cholesterol level, although CETP mass was reported to not correlate with HDL-cholesterol and correlate negatively with LDL-cholesterol in human plasma (Goto et al., 2001). These reports suggest that inhibition of plasma CETP activity may result in favorable changes in plasma lipoprotein profiles.

JTT-705, *S*-[2-([[1-(2-ethylbutyl)cyclohexyl]carbonyl]amino)phenyl]2-methylpropanethioate, is a synthetic benzenethiol derivative identified as a CETP inhibitor (Shinkai et al., 2000). Previously, we reported that JTT-705 suppresses CETP activity by forming a disulfide bond with cysteine at residue 13 of CETP, and in cholesterol-fed rabbits JTT-705 increased HDL-cholesterol and inhibited the progression of atherosclerosis (Okamoto et al., 2000). In this report, we investigated the effects of JTT-705 and its thiol form (JTP-25203), 1-(2-ethylbutyl)-cyclohexane carboxylic acid *N*-(2-mercaptophenyl) amide, on plasma CETP activities in several animal species, including humans, in vitro. We also orally administered JTT-705

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to normolipidemic rabbits, hamsters and marmosets, in order to investigate the HDL-cholesterol increasing effect of JTT-705 in plural animal species and predict efficacy in humans.

2. Materials and methods

2.1. Chemicals

JTT-705 and JTP-25203 were synthesized at the Central Pharmaceutical Research Institute of Japan Tobacco (JT) (Osaka, Japan).

2.2. Human and animal plasma

Human blood was obtained from healthy, normolipidemic volunteers recruited from the personnel of the Central Pharmaceutical Research Institute of JT. Blood was collected in tubes containing heparin (Terumo, Tokyo, Japan), and plasma was isolated by centrifugation at 1500 × *g* for 20 min at 4 °C.

All animal protocols complied with the guidelines for animal experimentation of our laboratories. Blood from male Japanese white rabbits (11–15 weeks old, Kitayama Labes, Nagano, Japan) was collected from the ear artery and blood from male Syrian hamsters (10–11 weeks old, Japan SLC, Shizuoka, Japan) was collected from the abdominal artery after anesthetizing with diethyl ether. All plasma was isolated by centrifugation at 1500 × *g* for 15 min at 4 °C. Plasma from male cynomolgus monkeys (body weight 4.10–6.66 kg) was purchased from Japan E.D.M. (Gifu, Japan), and plasma from male and female common marmosets (2–5 years old) was purchased from Mitsubishi Chemical Safety Institute (Ibaraki, Japan). For all blood collection, heparin solution was used as the anticoagulant.

2.3. Preparation of lipoprotein substrates

Lipoprotein substrates for CETP assay were prepared from human plasma by ultracentrifugation according to the modified method of Tollefson and Albers (1986). Human blood was collected in a blood bag containing 0.4% citric acid (Terumo), and plasma was isolated by centrifugation at 1500 × *g* for 20 min at 4 °C. Plasma was adjusted with solid potassium bromide (Wako, Osaka, Japan) to a density of 1.125 g/ml and centrifuged at 230,000 × *g* for 18 h. The bottom fraction was collected and was dialyzed against 10 mM phosphate-buffered saline containing 0.05% NaN_3 (Katayama Pure Chemical Industries, Osaka, Japan) and 1 mM EDTA (Nacalai Tesque, Kyoto, Japan) (pH 7.2). [1,2-^{3}H (N)] cholesterol (NEN™ Life Science Products, Boston, MA, USA) in 99% ethanol was added to the dialyzed fraction, and the mixture was incubated for 18 h at 37 °C. After the density was adjusted to 1.21 g/ml with potassium bromide, [^{3}H]cholesteryl ester-labeled HDL was isolated by ultracentrifugation (200,000 × *g* for 68–92 h). VLDL/LDL (1.006 g/ml < *d* < 1.063 g/ml) and HDL (1.063 g/ml < *d* < 1.21 g/ml) fractions were isolated from human plasma by serial ultracentrifugation at densities of 1.006 g/ml (at 230,000 × *g* for 4 h), 1.063 g/ml (at 230,000 × *g* for 18 h) and 1.21 g/ml (at 230,000 × *g* for 40 h). All isolated fractions were dialyzed against 10 mM phosphate-buffered saline containing 0.05% NaN_3 and 1 mM EDTA (pH 7.2). All substrates were stored at 4 °C after flushing with nitrogen.

2.4. Plasma CETP assay

JTT-705 and JTP-25203 were dissolved in a mixture of *N*-methyl-2-pyrrolidinone (NMP, Kanto Chemical Industries, Tokyo, Japan) and polyethylene glycol 400 (PEG, Wako) (1:1). Plasma from humans ($n = 10$; 8 males and 2 females), male rabbits ($n = 10$), male cynomolgus monkeys ($n = 6$), male hamsters ($n = 6$; each sample was the pooled plasma of 2 animals) and marmosets ($n = 3$; each sample was the pooled plasma of 2–12 animals) were used. Plasma was preincubated with compounds (final NMP/PEG concentration: 1%) for 4 h at 37 °C. After preincubation, aliquots of plasma (human: 4 μl, rabbit: 1.8 μl, hamster: 4.5 μl, cynomolgus monkey: 2.7 μl, marmoset: 3.6 μl; these plasma volumes contained similar CETP activity) were incubated for 15 h at 37 °C with [^{3}H]cholesteryl ester-labeled HDL (human, cynomolgus monkey and marmoset: 5.5 μg cholesterol; rabbit and hamster: 0.24 μg cholesterol), VLDL/LDL (human, cynomolgus monkey and marmoset: 354 μg cholesterol; rabbit and hamster: 23.8 μg cholesterol) in a total volume of 600 μl made up with Tris-buffered saline (pH 7.4) containing 0.1 mg/ml bovine serum albumin (Sigma). After precipitation of VLDL/LDL by dextran sulfate (final concentration: 0.027%) and

Table 1
Effects of JTT-705 and JTP-25203 on plasma CETP activities

Species	*n*	IC_{50} (μM)	
		JTT-705	JTP-25203
Human	10	5.5 ± 0.8	2.8 ± 0.4
Rabbit	10	1.0 ± 0.1	0.44 ± 0.03
Hamster (pooled plasma)	6	11.7 ± 1.7	0.52 ± 0.07
Cynomolgus monkey	6	2.4 ± 0.2	1.3 ± 0.1
Marmoset (pooled plasma)	3	6.3 ± 0.5	1.1 ± 0.3

After plasma was preincubated with compounds for 4 h at 37 °C, aliquots of plasma were mixed with [^{3}H]cholesteryl ester-labeled HDL and VLDL/LDL in a total volume of 600 μl made up with Tris-buffered saline containing 0.1 mg/ml bovine serum albumin, and incubated for 15 h at 37 °C. After precipitation of VLDL/LDL by dextran sulfate and $MgCl_2$, half of the supernatant was removed and counted in a liquid scintillation counter. The CETP activity was determined by the decrease in radioactivity versus that of a blank without plasma. The concentrations of compounds necessary to inhibit 50% of CETP activity (IC_{50}) were calculated. Each value represents the mean ± S.E.M.

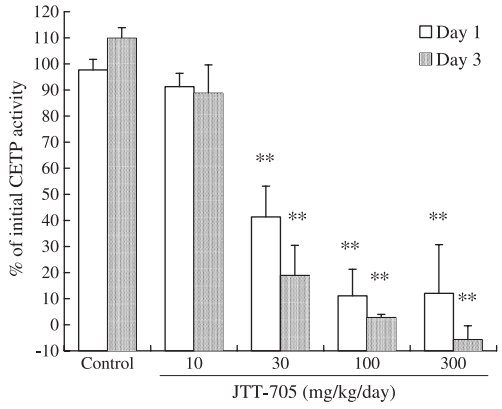

Fig. 1. Effect of JTT-705 on plasma CETP activity in JW rabbits. JTT-705 was administered orally at doses of 10, 30, 100 and 300 mg/kg once a day for 3 days. Plasma samples were taken before dosing and 2 h after dosing on days 1 and 3. CETP activity in plasma was determined as described in Materials and methods. Each value represents the mean ± S.E.M. $^{**}P < 0.01$, significantly different from control group by one-way ANOVA followed by Dunnett's test.

$MgCl_2$ (final concentration: 27 mM), half of the supernatant was removed and its radioactivity measured in a liquid scintillation counter (Wallac 1410, Pharmacia, Uppsala, Sweden). The CETP activity was determined from the decrease in radioactivity versus that of a blank without plasma.

2.5. Ex vivo and in vivo studies

This experiment complied with the guidelines for animal experimentation of our laboratories. Twenty four Japanese white rabbits (male, 8 weeks old, Kitayama Labes) and 39 Syrian hamsters (male, 10 weeks old, Japan SLC) were housed (temperature: 23 ± 3 °C; humidity: 55 ± 15%) with a light cycle of 8:00 am to 8:00 pm. Twenty common marmosets (male, 14–58 months old, Mitsubishi Chemical Safety Institute) were housed individually in single cages (temperature: 25 ± 3 °C; humidity: 53 ± 15%) with a light cycle of 8:00 am to 8:00 pm.

Rabbits were maintained on 100 g of standard chow during the experimental period, and divided into five groups, so as to make their plasma HDL-cholesterol and total cholesterol levels similar. JTT-705 was suspended in 0.5% methylcellulose (Shin-Etsu Chemical, Tokyo, Japan) solution, and orally administered to the rabbits at doses of 10, 30, 100 and 300 mg/kg body weight, once daily for 3

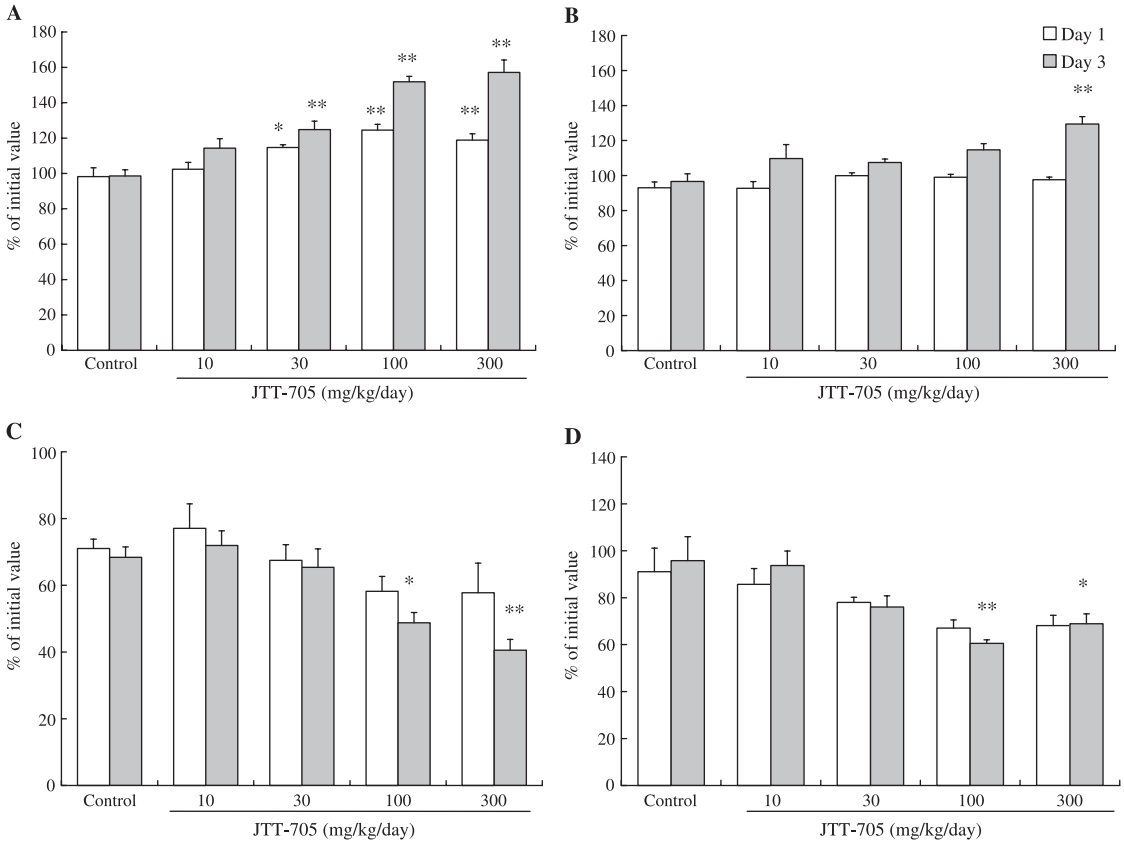

Fig. 2. Effect of JTT-705 on plasma lipid levels in JW rabbits. JTT-705 was administered orally at doses of 30, 100 and 300 mg/kg once a day for 3 days. Plasma samples were taken before dosing and 2 h after dosing on days 1 and 3, HDL-cholesterol (A), total cholesterol (B) and HDL-triglyceride (C) were measured using enzymatic assay and atherogenic index (D) was calculated as [{(total cholesterol) − (HDL-cholesterol)}/(HDL-cholesterol)]. The percentage of each lipid (*y*-axis) was calculated as [(value at each time point/value at initial time point) × 100]. Each value represents the mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from control group by one-way ANOVA followed by Dunnett's test.

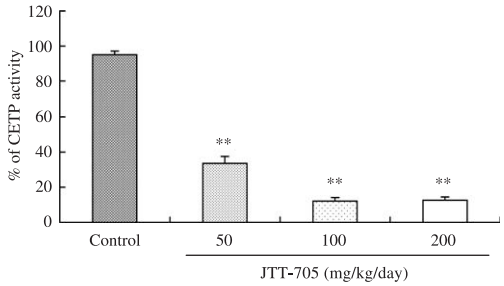

Fig. 3. Effect of JTT-705 on plasma CETP activity in Syrian hamsters. JTT-705 was administered orally at doses of 50, 100 and 200 mg/kg. Plasma samples were taken 2 h after dosing and CETP activity in plasma was determined as described in Materials and methods. Each value represents the mean ± S.E.M. $^{**}P < 0.01$, significantly different from control group by one-way ANOVA followed by Dunnett's test.

days. Blood samples were obtained from the ear artery before dosing, 2 and 7 h after dosing on days 1 and 3.

Hamsters were maintained ad libitum on standard chow, and were divided into four groups, so as to make their plasma HDL-cholesterol and total cholesterol levels similar. JTT-705 was orally administered at doses of 50, 100 and 200 mg/kg body weight, once daily for 7 days. Blood samples were obtained from the supraorbital vein 1 day before dosing, 2 h after first dosing and 7 h after final dosing.

Marmosets were maintained on 80 g/day of standard chow CMS-1 (Clea Japan, Tokyo, Japan) and divided into four groups, so as to make their serum HDL-cholesterol and total cholesterol levels similar. JTT-705 was orally administered at doses of 10 or 30 mg/kg three times a day (30 or 90 mg/kg/day), or 30 mg/kg twice a day (60 mg/kg/day) for 28 days. The soluble chow of 14% (w/v) CMS-1 (2 ml/animal) was administered simultaneously at each JTT-705 dosing. Blood samples were obtained from the femoral vein 3 days before dosing, 2 h after last dosing on days 1, 13 and 28 and 14 h after final dosing on days 7, 14, 21 and 28.

Plasma (rabbits and hamsters) or serum (marmosets) was isolated by centrifugation at 11,000 × *g* for 5 min at 4 °C. HDL fractions were taken after precipitation of apolipoprotein B (apoB)-containing lipoproteins with 13% polyethylene glycol 6000 (Wako) (Chiba et al., 1997). Total cholesterol, triglyceride, HDL-cholesterol and HDL-triglyceride levels in plasma or serum were determined enzymatically using commercially available kits (Boehringer Mannheim, Mannheim, Germany) with a Cobas FARA II analyzer (Roche, Basel, Switzerland). Non-HDL-cholesterol and atherogenic index were calculated as [(total cholesterol) − (HDL-cholesterol)] and [(non-HDL-cholesterol)/(HDL-cholesterol)], respectively. CETP activity in plasma (rabbit: 1.8 μl, hamster: 4 μl) or serum (marmoset: 3.6 μl) was determined as above.

Lipoproteins in marmoset serum taken 14 h after final administration on day 28 were analyzed by agarose gel electrophoresis. Aliquots of 1 μl (cholesterol staining) or 3 μl (triglyceride staining) of serum were electrophoresed in 1% agarose gel film (Titan Gels, Helena Laboratories, Beaumont, TX) at 90 V for 25 min. After drying, the gels

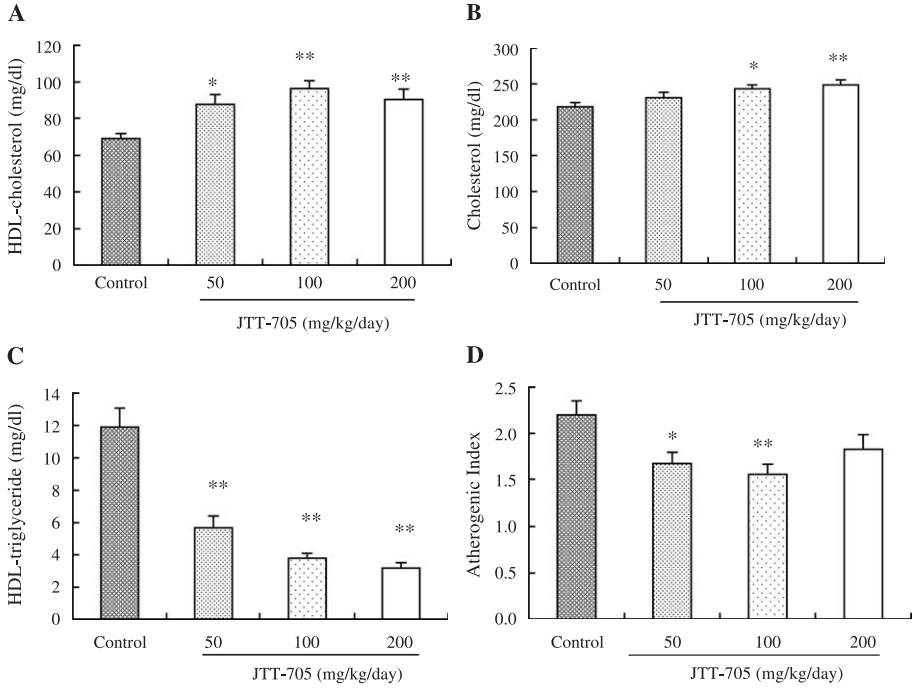

Fig. 4. Effect of JTT-705 on plasma lipid levels in JW rabbits. JTT-705 was administered orally at doses of 50, 100 and 200 mg/kg once a day for 7 days. Plasma samples were taken 7 h after last dosing, HDL-cholesterol (A), total cholesterol (B) and HDL-triglyceride (C) were measured using enzymatic assay and atherogenic index (D) was calculated. Each value represents the mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from control group by one-way ANOVA followed by Dunnett's test.

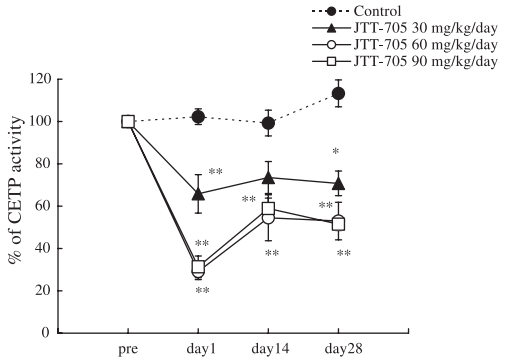

Fig. 5. Effect of JTT-705 on serum CETP activity in common marmosets. JTT-705 was administered orally at doses of 10 and 30 mg/kg three times a day (30 and 90 mg/kg/day, respectively) or 30 mg/kg twice a day (60 mg/kg/day) for 28 days. Serum samples were taken 3 days before dosing (pre) and 2 h after last dosing on days 1, 14 and 28. CETP activities in serum were determined as described in Materials and methods. Each value represents the mean ± S.E.M. $*P<0.05$, $**P<0.01$, significantly different from control group by one-way ANOVA followed by Dunnett's test.

were stained with Titan Gel S-cholesterol or S-triglyceride (Helena Laboratories). After destaining the gels they scanned by a densitometer (CS-9300PC, Shimadzu, Kyoto, Japan). The total amount of cholesterol in α- and slow α-lipoprotein was calculated as follows: [{(peak area of α-lipoprotein)+(peak area of slow α-lipoprotein)}/(total peak area)] × (serum TC).

2.6. Statistical analysis

All values were expressed as mean ± S.E.M. Comparisons among multi-groups in the ex vivo and in vivo studies were made using one-way analysis of variance (ANOVA) followed by Dunnett's two-tailed test. P values less than 0.05 were considered significant. All statistical analyses were performed using Super ANOVA software (Abacus Concepts, Berkeley, CA, USA) and StatLight software (Yukms, Tokyo, Japan).

3. Results

3.1. Effect on plasma CETP activities in human and animals

JTT-705 and JTP-25203 inhibited plasma CETP activities in humans, rabbits, hamsters, cynomolgus monkeys and marmosets. For these species, IC_{50} values for JTT-705 were 5.5 ± 0.8, 1.0 ± 0.1, 11.7 ± 1.7, 2.4 ± 0.2 and 6.3 ± 0.5 μM, respectively, while IC_{50} values for JTP-25203 were 2.8 ± 0.4, 0.44 ± 0.03, 0.52 ± 0.07, 1.3 ± 0.1 and 1.1 ± 0.3 μM, respectively (Table 1).

3.2. Effect on plasma CETP activities and lipid levels in rabbits by oral administration

Before administration of JTT-705, the levels of HDL-cholesterol, total cholesterol, and HDL-triglyceride in rabbit plasma were 15.03 ± 0.87, 37.17 ± 1.40 and 20.94 ±0.76 mg/dl, respectively, and atherogenic index was 1.56 ±0.10.

JTT-705 was orally administered to the rabbits at doses of 10, 30, 100 and 300 mg/kg once daily for 3 days. JTT-705 reduced plasma CETP activity significantly at the doses of more than 30 mg/kg/day on days 1 and 3 (Fig. 1). JTT-

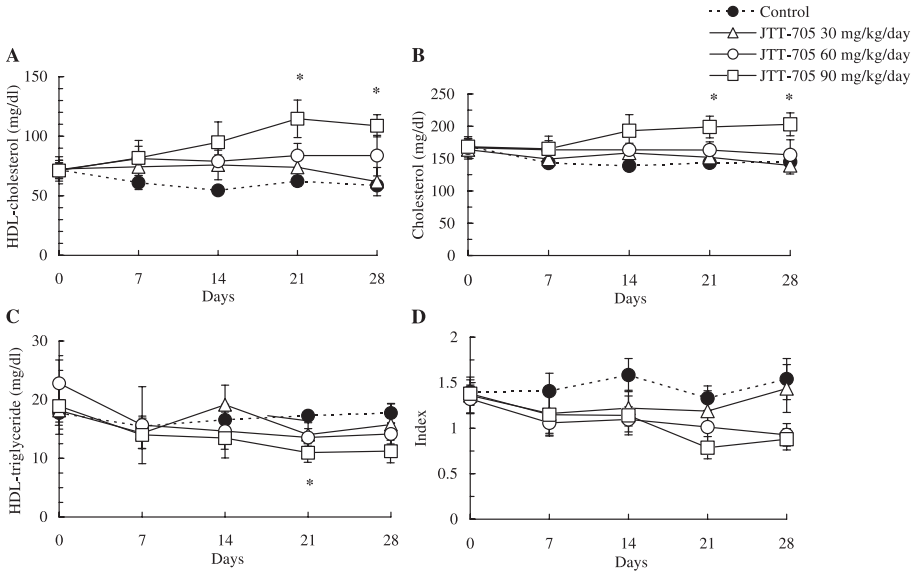

Fig. 6. Effect of JTT-705 on serum lipid levels in common marmosets. JTT-705 was administered orally at doses of 10 and 30 mg/kg three times a day (30 and 90 mg/kg/day, respectively) or 30 mg/kg twice a day (60 mg/kg/day) for 28 days. Serum samples were taken 3 days before dosing (pre) and 14 h after last dosing on days 1, 7, 14 and 28 (0, 7, 14 and 28 days). Serum HDL-cholesterol (A), total cholesterol (B) and HDL-triglyceride (C) were measured using enzymatic assay and atherogenic index (D) was calculated. Each value represents the mean ± S.E.M. $*P<0.05$, significantly different from control group by one-way ANOVA followed by Dunnett's test.

705 increased HDL-cholesterol by 16%, 27%, 54% and 59%, and decreased atherogenic index by 2%, 21%, 37% and 28% at the doses of 10, 30, 100 and 300 mg/kg/day, respectively, on day 3 (Fig. 2). HDL-triglyceride was decreased by 4%, 29% and 41% in the JTT-705 30, 100 and 300 mg/kg/day groups, respectively, on day 3 (Fig. 2). Although JTT-705 at 300 mg/kg/day increased total cholesterol by 34% on day 3(Fig. 2), JTT-705 did not affect non-HDL-cholesterol and triglyceride levels (data not shown).

3.3. Effect on plasma CETP activities and lipid levels in hamsters by oral administration

JTT-705 was orally administered to normolipidemic hamsters once daily for 7 days. Plasma CETP activity was significantly reduced by 66%, 87% and 87% at the 50, 100 and 200 mg/kg/day JTT-705, respectively, 2 h after first dosing (Fig. 3). Seven hours after final dosing, HDL-cholesterol was increased by 27%, 40% and 31%, HDL-triglyceride was decreased by 53%, 68% and 73%, and atherogenic index was decreased by 24%, 29% and 17% in the JTT-705 50, 100 and 200 mg/kg/day groups, respectively (Fig. 4). Although JTT-705 at 100 and 200 mg/kg/day increased plasma total cholesterol significantly, JTT-705 did not affect non-HDL-cholesterol and triglyceride levels (data not shown).

3.4. Effect on serum CETP activities and lipid levels in marmosets by oral administration

JTT-705 at 30, 60 and 90 mg/kg/day reduced CETP activity by 34%, 71% and 69%, respectively, 2 h after last dosing on day 1, and by 29%, 47% and 49%, respectively, 2 h after last dosing on day 28 (Fig. 5). JTT-705 maximally increased HDL-cholesterol by 31%, 45% and 81%, decreased HDL-triglyceride by 12%, 37% and 42%, and decreased atherogenic index by 23%, 38% and 44% at the doses of 30, 60 and 90 mg/kg/day, respectively (Fig. 6). Although 90 mg/kg/day JTT-705 increased serum total cholesterol significantly, it did not affect non-HDL-cholesterol and triglyceride levels (data not shown).

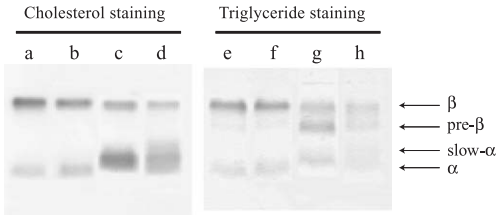

Fig. 7. Electrophoretic analysis of serum lipoproteins in marmosets using agarose gel film. Serum samples were taken 14 h after last dosing on day 28. Aliquots of 1 μl (cholesterol staining) or 3 μl (triglyceride staining) of serum were electrophoresed in 1% agarose gel film at 90 V for 25 min. After drying, the gels were stained with Titan Gel S-cholesterol or S-triglyceride. Lanes a, b, c and d: cholesterol staining; lanes e, f, g and h: triglyceride staining; lanes a and e: control; lanes b and f: JTT-705 30 mg/kg/day; lanes c and g: JTT-705 60 mg/kg/day; lanes d and h: JTT-705 90 mg/kg/day.

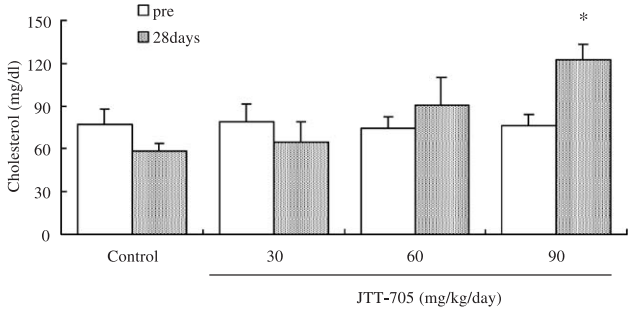

Fig. 8. Effect of JTT-705 on cholesterol contents in α- and slow α-lipoprotein. Serum samples were taken 3 days before dosing (pre) and 14 h after last dosing on day 28 (28 days). Aliquots of 1 μl of serum were electrophoresed in 1% agarose gel film at 90 V for 25 min. After drying, the gels were stained with Titan Gel S-cholesterol. After the gels were destained, they were scanned by a densitometer. The total amount of cholesterol in α- and slow α-lipoprotein was determined as described in Materials and methods. Each value represents the mean ± S.E.M. $*P < 0.05$, significantly different from control group by one-way ANOVA followed by Dunnett's test.

In the analysis of serum lipoproteins using agarose gel electrophoresis, 90 mg/kg/day JTT-705 increased slow α-migrating lipoprotein (Fig. 7). Cholesterol content in HDL (slow α- and α-lipoproteins) was increased by 11%, 56% and 110% at doses of 30, 60 and 90 mg/kg/day, respectively (Fig. 8).

After dosing for 28 days, there was no difference in body weight between the control group and JTT-705-treated groups (control: 325.4 ± 9.7 g, JTT-705 30 mg/kg/day: 352.0 ± 18.4 g, JTT-705 60 mg/kg/day: 342.4 ± 10.6 g and JTT-705 90 mg/kg/day: 346.2 ± 11.0 g).

4. Discussion

Human CETP contains seven cysteine residues (Drayna et al., 1987), and we reported that cysteine at residue 13 of CETP appears essential for inhibitory activity of JTT-705 (Okamoto et al., 2000). Since another CETP inhibitor, SC-71952, was reported to bind to the same cysteine residue (Hope et al., 2000), this suggests that the cysteine at residue 13 is near a hydrophobic lipid binding site and that these compounds disturb the binding of cholesteryl ester and/or triglyceride with CETP. As JTT-705 inhibited plasma CETP activities of humans, rabbits, hamsters, cynomolgus monkeys and marmosets, the tertiary structure around the cysteine may be conserved among these animal species. JTT-705 may bind to the cysteine via a disulfide bond, therefore a thiol form (JTP-25203) of JTT-705 is expected to be an active inhibitor of CETP. In fact, JTT-705 is hydrolyzed to the thiol form in plasma or neutral buffer, and the inhibitory activity of JTP-25203 is superior to that of JTT-705 in vitro. The IC_{50} values of JTT-705 were 2-fold (human, rabbit and cynomolgus monkey), 6-fold (marmoset) or 20-fold (hamster) higher than those of JTP-25203. These results suggest that the efficiency of conversion from

JTT-705 to the thiol form in plasma may be different between animal species, especially hamsters that have the low plasma CETP levels (Bisgaier et al., 1993). As the IC_{50} values of JTP-25203 were similar among different animal species and JTT-705 can be almost completely hydrolyzed to the thiol form in vivo, orally administered JTT-705 is thought to similarly inhibit CETP activity in a wide range species, from rodents to primates.

As thiol compounds are generally unstable, we subjected orally bioavailable thioester, JTT-705, to in vivo studies. Oral administration of JTT-705 inhibited plasma CETP activity, increased plasma HDL-cholesterol and decreased atherogenic index in rabbits, hamsters and marmosets. In rabbits and hamsters, 100 mg/kg/day JTT-705 suppressed plasma CETP activity almost completely, elevated HDL-cholesterol by 54% and 40%, and reduced atherogenic index by 37% and 29%, respectively. In the marmoset study, 90 mg/kg/day JTT-705 for 28 days reduced plasma CETP activity by 69%, elevated HDL-cholesterol by 81%, and reduced atherogenic index by 44%. Although the potency of ex vivo CETP inhibition in marmosets was weaker than those in rabbits and hamsters, the extent of plasma HDL-cholesterol increase could be higher by means of long-term dosing of JTT-705. Moreover, JTT-705 increased slow α-migrating lipoprotein in marmoset serum. Since the slow α-migrating lipoprotein is reported to be the same as apolipoprotein E-rich HDL (HDLc) and to be present in plasma from humans with CETP deficiency (Chiba et al., 1997), HDL metabolism in JTT-705-treated marmosets seems to be similar to that in CETP-deficient humans. These results show that JTT-705 has potential in increasing plasma HDL-cholesterol in a wide range of animal species, including humans. Recently, HDL-cholesterol increasing effect of JTT-705 in humans was demonstrated in a clinical study using healthy subjects with mildly elevated LDL-cholesterol (De Grooth et al., 2002).

Many studies demonstrate that HDL-cholesterol is an antiatherogenic factor (Von Eckardstein et al., 2001; Nofer et al., 2002; Yuhanna et al., 2001), and low HDL-cholesterol in plasma increases the risk for coronary heart desease (Corti et al., 1995; Barter and Rye, 1996). Genetic CETP deficiency shows high plasma HDL-cholesterol, but a possible role of CETP in atherogenesis is still being debated. Several epidemiological studies showed genetic CETP deficients have a high risk for atherosclerosis (Hirano et al., 1995, 1997; Zhong et al., 1996; Bruce et al., 1998; Agerholm-Larsen et al., 2000), although in CETP polymorphism TaqIB the B2 allele is reported to reduce the risk of coronary heart desease (Kuivenhoven et al., 1998; Ordovas et al., 2000). Similarly, in CETP-transgenic mice, CETP appears to be either proatherogenic (Marotti et al., 1993; Plump et al., 1999) or antiatherogenic (Hayek et al., 1995; Foger et al., 1999). In contrast, CETP is suggested to accelerate the progression of atherosclerosis in the cholesterol-fed rabbit model. Suppression of CETP expression by antisence oligonucleotide and immunological inhibition of CETP by vaccination with CETP peptide were reported to inhibit CETP activity, improve lipoprotein profile and prevent the progression of atherosclerosis in cholesterol-fed rabbits (Sugano et al., 1998; Rittershaus et al., 2000). We also demonstrated that CETP inhibition by JTT-705 raised plasma HDL-cholesterol, and prevented the progression of atherosclerosis in 0.2% cholesterol-fed rabbits (Okamoto et al., 2000). As for the anti-atherogenic mechanism of JTT-705, it is possible that JTT-705 would accelerate the reverse transport of cholesterol from the arterial wall to the liver through the increases of HDL_3-cholesterol and apolipoprotein A-I (Okamoto et al., 2000). In fact, it was recently reported that HDL from JTT-705-administered rabbits was able to reduce cholesteryl ester concentration in J774 macrophage as efficiently as that from control rabbits (Kobayashi et al., 2002), suggesting that the absolute HDL concentration increased by JTT-705 would suppress the lipid deposition within the arterial wall. Our present study shows that JTT-705 can inhibit plasma CETP activity and increase HDL-cholesterol without difference among animal species. Moreover, the efficacy of JTT-705 in the clinical study (de Grooth et al., 2002) is similar to that in the cholesterol-fed rabbits (Okamoto et al., 2000) from the viewpoint of HDL-cholesterol increase and non-HDL-cholesterol (LDL-cholesterol) decrease. These results suggest the possibility that the anti-atherogenic effect of JTT-705 in rabbits can also be extrapolated to other animal species, including humans.

In conclusion, JTT-705 inhibited plasma CETP activities and increased plasma HDL-cholesterol resulting in antiatherogenic changes in lipoprotein profiles in all animal species that were examined in this study. JTT-705 is potentially useful for the treatment of hyperlipidemia and hypoalphalipoproteinemia.

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