Pharmacological properties of a novel ACAT inhibitor (CP-113,818) in cholesterol-fed rats, hamsters, rabbits, and monkeys

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Abstract The novel acyl-CoA:cholesterol acyltransferase (ACAT) inhibitor CP-113,818 has been characterized in vitro against ACAT isolated from liver and intestine from a variety of species including human subjects, and in vivo in cholesterol-fed rats, hamsters, rabbits, and two species of nonhuman primates. CP-113,818 is a potent and specific inhibitor of liver and intestinal ACAT with IC_{50}s ranging from 17 to 75 nM. This ACAT inhibitor also prevented the absorption of exogenous radiolabeled cholesterol in hamsters (ED_{50} = 6 μg/kg), rabbits (ED_{50} ½ 10 μg/kg), and cynomolgus and African green monkeys (40 and 26% inhibition at 10 mg/kg, respectively). CP-113,818 effectively prevented the increase in liver cholesterol levels in cholesterol-fed rats, hamsters, and rabbits. In lipoprotein characterization studies in rabbits, CP-113,818 selectively decreased apoB-containing lipoproteins (β-VLDL, IDL, and LDL) and shifted the distribution of cholesterol from β-VLDL, IDL, and LDL (96% before treatment to 81% after treatment) to HDL (4% before treatment to 19% after treatment). Finally, in monkeys, CP-113,818 significantly decreased LDL cholesterol by approximately 30% while either increasing HDL cholesterol (cynomolgus monkeys) or not affecting HDL cholesterol (African green monkeys), thereby improving the total plasma cholesterol/HDL ratios. In summary, CP-113,818 significantly inhibited cholesterol absorption, prevented the increase in liver cholesterol, and improved the lipoprotein profiles by selectively decreasing the cholesterol concentrations of the atherogenic lipoproteins (VLDL, IDL, and LDL) in a variety of cholesterol-fed animals. These data suggest that ACAT inhibition may be a useful therapeutic approach for lowering LDL cholesterol and thereby reducing the risk of developing coronary heart disease.—**Marzetta, C. A., Y. E. Savoy, A. M. Freeman, C. A. Long, J. L. Pettini, R. E. Hagar, P. B. Inskeep, K. Davis, A. F. Stucchi, R. J. Nicolosi, and E. S. Hamanaka.** Pharmacological properties of a novel ACAT inhibitor (CP-113,818) in cholesterol-fed rats, hamsters, rabbits, and monkeys. *J. Lipid Res.* 1994. **35:** 1829–1838.

Supplementary key words cholesteryl ester • TPC • LDL • HDL • cholesterol absorption

The regulation of plasma cholesterol levels is complex and involves factors that influence both extracellular as well as intracellular cholesterol metabolism. Intracellularly, acyl coenzyme A:cholesterol acyl transferase (ACAT; EC2.3.1.26) has been identified as a key enzyme in cholesterol homeostasis (1). ACAT is the major cholesterol esterifying enzyme found in the intestine, liver, and artery wall, the major organs involved in cholesterol metabolism and atherosclerosis.

In the intestine, ACAT activity is highest in the proximal jejunum and lower in the proximal duodenum and distal ileum, corresponding to the predominant site of cholesterol absorption (2). Intestinal ACAT is involved in the absorption of cholesterol and its subsequent secretion as esterified cholesterol in chylomicrons (3).

In the liver, ACAT activity is important in lipoprotein cholesterol production and secretion (4, 5). That is, stimulating ACAT activity has been shown to increase very low density lipoprotein (VLDL) cholesteryl ester secretion in cultured rat hepatocytes (4), and inhibiting ACAT activity has been shown to decrease VLDL and LDL secretion in perfused monkey livers (5). Recently, liver ACAT activity was shown to be significantly correlated to plasma LDL cholesteryl ester enrichment and coronary artery atherosclerosis in cholesterol-fed African green monkeys (6).

Abbreviations: ACAT, acyl-CoA:cholesterol acyltransferase; LDL, low density lipoprotein; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein; DGUC, density gradient ultracentrifugation; TPC, total plasma cholesterol.

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Finally, the association between arterial ACAT activity and atherogenesis has been recognized for over 20 years. Studies in pigeons, rabbits, pigs, and monkeys have shown that arterial ACAT activity increases up to 70-fold with cholesterol feeding and the subsequent development of atherosclerotic lesions (7–11). Recently, in a cholesterol-fed and injury-induced model of atherosclerosis in rabbits and Yucatan pigs, an ACAT inhibitor was shown to prevent the accumulation of foam cells and to prevent the further increase of arterial cholesteryl ester content without increasing arterial free cholesterol (12, 13). This has been an important observation as cholesteryl ester deposition is the hallmark of the atherosclerotic lesion (14).

As intestinal ACAT plays an important role in cholesterol absorption, as hepatic ACAT influences lipoprotein secretion, and as arterial ACAT is important in cholesteryl ester deposition in atherogenesis, inhibiting ACAT may be a viable therapeutic approach for decreasing plasma cholesterol concentrations and decreasing the development of coronary heart disease in hypercholesterolemic subjects. Therefore, we have investigated the effects of a novel ACAT inhibitor, CP-113,818, on in vitro ACAT inhibition and on cholesterol absorption, liver cholesterol, and lipoprotein cholesterol in a variety of cholesterol-fed animal models.

MATERIALS AND METHODS

CP-113,818 is (−)-*N*-(2,4-bis(methylthio)-6-methylpyridin-3-yl)-2-(hexylthio) decanoic amide (**Fig. 1**) (15). The free base (CP-113,818) or the chloride salt form (CP-113,818-01) were used throughout these studies. As no differences in in vitro or in vivo activity have been seen with either form, the compound is referred to as CP-113,818 for clarity. [1α,2α(n)-^{3}H]cholesterol (52 mCi/mmol) and [1-^{14}C]oleoyl coenzyme A (52.7 mCi/mmol) were obtained from Amersham.

In vitro ACAT activity assay

Liver samples were obtained from chow-fed rats and cholesterol-fed hamsters (0.2% cholesterol, 0.1% cholic acid diet; 16), rabbits (0.2% cholesterol with 11% of calories as peanut oil), cynomolgus monkeys (0.1% cholesterol with 42% of calories as lard; 17) and African green monkeys (0.2% cholesterol with 42% of calories as lard; diet shown in ref. 18 but with half the cholesterol). Intestinal mucosal scrapings from the ileum and jejunum were obtained from cholesterol-fed hamsters, rabbits, cynomolgus monkeys, African green monkeys, and two normolipidemic human subjects. The human tissues were obtained from non-cadaverous, normal subjects through the services of the International Institute for the Advancement of Medicine.

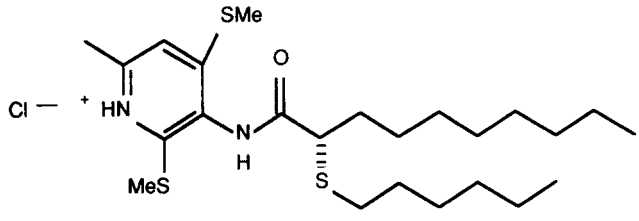

Fig. 1. Chemical structure of CP-113,818; $C_{24}H_{42}N_2OS_3 \cdot HCl$; 507.26 g/mole.

Microsomes were prepared from liver and intestinal mucosal scrapings by sequential centrifugation and in vitro ACAT activity assays were done according to the method of Billheimer (19). Briefly, 100 μg microsomal protein, 22 μg BSA, and 52 nmol cholesterol ± CP-113,818 in 5 μl DMSO were preincubated for 30 min at 37°C in a phosphate buffer (200 μl total volume). After 30 min, 1 nmol [^{14}C]oleoyl-CoA was added as substrate and incubated for an additional 20 min. The reaction was stopped with the addition of 1 ml ethanol and lipids were extracted with hexane. Cholesteryl [^{14}C]oleate formation was quantified by thin-layer chromatography and data are expressed as percent inhibition of ACAT activity (pmol/μg protein per min) compared to a control sample incubated with no drug. All samples were run in duplicate.

Cholesterol absorption in rats, hamsters, rabbits, and monkeys

Male Sprague-Dawley rats were fed (ad libitum) a diet (US Biochemicals; AIN #76A; Cleveland, OH) supplemented with 1% cholesterol and 0.5% cholic acid and gavaged daily with either CP-113,818 (1, 30, or 150 mg/kg) dissolved in a saline solution of 10% ethanol, 0.25% methyl cellulose, and 0.6% Tween-80 or the ethanol/methyl cellulose/Tween-80 vehicle alone for the control group. After 13 days of treatment, each animal received 2 μCi [^{3}H]cholesterol by gavage in a liquid diet (BioServ F-0739; Frenchtown, NJ) and were euthanized 24 h later. The amount of radiolabeled cholesterol in the plasma and liver of each animal was quantified and used to estimate the amount of [^{3}H]cholesterol absorbed by the method of Kelley and Tsai (20) and modified as described by Harwood et al. (16). Data are expressed as the percent inhibition of cholesterol absorbed compared to the untreated controls. Average percentage of the radiolabeled cholesterol absorbed in untreated control rats was 34.0 ± 3% (mean ± SD; n = 5; plasma + liver radioactivity).

Male Golden Syrian hamsters were fed (ad libitum) a diet (AIN #76A) containing 1% cholesterol and 0.5% cholic acid for 4 days. After an overnight fast, each animal received a bolus of CP-113,818 (3, 10, 30, 100, or 300 μg/kg) dissolved in a saline solution of 10% ethanol, 0.25% methyl cellulose, and 0.6% Tween-80 by gavage (or the vehicle alone for the control group) followed immediately by an oral bolus of liquid hamster diet containing 2 μCi of [^{3}H]cholesterol. Twenty four hours later the

animals were euthanized and the amount of [^{3}H]cholesterol absorbed was estimated by quantifying liver and plasma [^{3}H]cholesterol. Liver samples were homogenized in saline (2 ml/g liver) with a Polytron homogenizer. Radioactivity was determined by counting aliquots of liver homogenate and plasma that were treated (bleached) with 30% H_2O_2. Total amount of [^{3}H]cholesterol absorbed was calculated and expressed as the percent inhibition of cholesterol absorbed compared to an untreated control group as described previously. Average percentage of the radiolabeled cholesterol absorbed in untreated control hamsters was 32.2 ± 3% (mean ± SD; n = 16; plasma + liver radioactivity).

Male New Zealand White (NZW) rabbits were fed a rabbit chow diet (Teklad TD-89021; Madison, WI) containing 0.4% cholesterol with 11% of calories as peanut oil for 1 week (meal-fed 85 g of diet once a day). The animals were then given CP-113,818 (0.025, 0.1, or 0.5 mg/kg) once a day orally (or vehicle alone for the control group) followed immediately by their daily meal. On the fifth day of drug treatment, each animal was given 15 μCi [^{3}H]cholesterol orally (dissolved onto the gelatin vehicle) along with the usual dose of drug or vehicle, followed by the meal. Three days later, the animals were bled and the amount of ^{3}H radioactivity in the plasma compartment was quantified and used to estimate the amount of [^{3}H]cholesterol that was absorbed. The size of the plasma compartment was estimated as 3.8% of body weight (21). Data are expressed as the percent inhibition of [^{3}H]cholesterol absorbed compared to the untreated control animals, as described previously. Average percentage of the radiolabeled cholesterol absorbed in untreated control rabbits was 18.2 ± 9% (mean ± SD; n = 4; plasma radioactivity only).

Eleven male cynomolgus macaques (*Macaca fascicularis*) were fed a diet containing 0.22 mg of cholesterol/kcal with 42% of calories as fat (polyunsaturated/saturated fat ratio = 0.45) for over 6 months prior to the start of this study (meal-fed twice a day). The animals were randomized into two groups on the basis of their total plasma cholesterol concentrations and were then given 10 mg/kg per day (n = 6) or 30 mg/kg per day (n = 5) of CP-113,818 b.i.d. on a piece of fruit. Immediately after consumption of the piece of drug-treated fruit, the animals received their usual meal. A cholesterol absorption study was done on day 22 of the drug treatment period using the dual isotope method of Zilversmit (22). Each animal received 60 μCi [^{3}H]cholesterol in a dextrin-containing gelatin capsule orally (using a pill gun) and 30 μCi [^{14}C]cholesterol suspended in 1 ml homologous serum intravenously (saphenous vein). Plasma samples were obtained 96 h later and the percent cholesterol absorption was calculated as described by Zilversmit (22). There were no statistically significant differences in any measured parameter between the 10 mg/kg and the 30 mg/kg doses, therefore, the data presented are for both doses combined. Average percentage of radiolabeled cholesterol absorbed in untreated control animals was 81.1 ± 3 (mean ± SD; n = 5).

Eighteen male and female African green monkeys (*Cercopithecus aethiops*) were fed a diet containing 0.4 mg cholesterol/kcal with 35% of calories as fat (polyunsaturated/saturated fat ratio = 0.32) for over 12 months prior to the start of this study (meal-fed twice a day). One week before the start of drug treatment, a cholesterol absorption study was done. Each animal received 5 μCi [^{14}C]β-sitosterol and 2.8 μCi of [^{3}H]cholesterol. Feces were collected and pooled from each animal for the next 4 days. Each fecal sample was homogenized with water, then saponified, and lipids were extracted with hexane. Aliquots of hexane were bleached under a UV light and the amount of radioactivity was determined in a liquid scintillation spectrometer. Cholesterol absorption was calculated as the percent of ingested dose according to the method of Borgström (23). The following week, the animals were randomized into three groups on the basis of their total plasma cholesterol concentrations and then given vehicle only (n = 6), 10 mg/kg per day (n = 6) or 30 mg/kg per day (n = 6) of CP-113,818 b.i.d. on a piece of fruit. A cholesterol absorption study was repeated on day 22 of the drug treatment period as described above. There were no differences in any parameter measured between the 10 mg/kg and the 30 mg/kg doses; therefore, except where noted, the data presented are for both doses combined. Average percentage of radiolabeled cholesterol absorbed in untreated control animals was 41.6 ± 6 (mean ± SD; n = 6).

Chronic effect of CP-113,818 on cholesterol absorption

Thirty-six male Golden Syrian hamsters were fed (ad libitum) a diet containing 0.2% cholesterol and 0.1% cholic acid with 22% of calories as safflower oil for 4 days. The animals were then given either CP-113,818 or vehicle once a day by gavage for up to 2 weeks. Groups of three drug-treated and three control animals were given 2 μCi [^{3}H]cholesterol by gavage on days 1, 4, 8, 11, and 14, euthanized 24 h later, and cholesterol absorption was estimated as described previously.

Fifteen male NZW rabbits were fed a diet containing 0.4% cholesterol with 11% of calories as peanut oil for 1 week (meal-fed once a day). The animals were then given 0.5 mg/kg per day once a day orally in a gelatin vehicle as described before and five rabbits were given the vehicle only. After 1 week of treatment, subsets of five CP-113,818-treated animals and five control animals were given an oral bolus of [^{3}H]cholesterol and cholesterol absorption was estimated as described previously. After 2 weeks of treatment, cholesterol absorption was determined in another subset of five CP-113,818-treated animals. Finally, after 3 weeks of drug treatment

cholesterol absorption was determined in the final subset of CP-113,818-treated animals.

Plasma, lipoprotein, and liver cholesterol and triglyceride determinations

Plasma was isolated from blood samples by centrifugation at 2700 rpm for 20 min at 4°C. Total plasma cholesterol (TPC) and lipoprotein cholesterol concentrations were measured enzymatically by the method of Auerbach, Parks, and Applebaum-Bowden (24) using commercially available reagents (Wako Pure Chemical Industries) and standards (Boehringer Mannheim). Plasma triglycerides were measured enzymatically using the Triglyceride Test Kit (Wako).

HDL cholesterol was measured according to the method of Assmann et al. (25). VLDL was isolated from plasma by ultracentrifugation in a 50.4 rotor (Beckman; Palo Alto, CA) at 40,000 rpm for 18 h at 10°C (26). In rabbit studies, LDL + IDL cholesterol was calculated by subtracting VLDL and HDL cholesterol from total plasma cholesterol.

Total plasma and HDL cholesterol concentrations were measured for both species of nonhuman primates using standardized Lipid Research Clinic (LRC) methodology (27, 28). Plasma VLDL + LDL (V + LDL) cholesterol was calculated by subtracting HDL cholesterol from TPC. Plasma triglycerides were measured enzymatically using an LRC standardized assay (29).

Liver samples were obtained immediately after each animal was euthanized and homogenized in 2 volumes of saline using a Polytron homogenizer. Aliquots were taken and the lipids were extracted with ethanol and hexane; the phases were split with H_2O, and an aliquot of the hexane phase was transferred to another tube and dried under N_2 at room temperature. The dried lipids were resolubilized in 1% Triton X-100 in ethanol, sonicated, and assayed for cholesterol using an enzymatic cholesterol assay (24).

Changes in lipoprotein cholesterol and protein distribution in rabbits

The distribution of protein among the IDL and LDL particles was characterized by density gradient ultracentrifugation (DGUC) by a modified method of Marzetta, Foster, and Brunzell (26). Briefly, VLDL was removed from plasma by ultracentrifugation and the density of 2.5 ml of the remaining d > 1.006 g/ml plasma fraction from each animal was raised to 1.21 g/ml with KBr. Discontinuous salt gradients were set up by underlayering the following solutions into ultracentrifuge tubes: 4.3 ml of a 1.006 g/ml solution, 5 ml of a 1.030 g/ml solution, and 2.6 ml (including the plasma sample) of a 1.21 g/ml solution. The samples were then ultracentrifuged at 41,000 rpm for 24 h at 15°C in an SW-41 rotor (Beckman; Palo Alto, CA). After ultracentrifugation, each sample was drained through a flow-cell UV monitor and a relative protein profile was obtained from recording absorbance at 280 nM. VLDL was shown to be primarily β-VLDL by agarose electrophoresis (Beckman Lipogel).

Plasma concentrations of CP-113,818

Plasma concentrations of CP-113,818 were determined by HPLC (P. B. Inskeep, W. E. Ballinger, J. T. Mayne, A. G. Connolly, and K. M. Davis, unpublished results).

Unpaired or paired *t*-tests or regression analyses were used for statistical analyses as stated. The *t*-tests were corrected for multiple tests by the method of Bonferoni (30).

RESULTS

The effect of CP-113,818 on intestinal and hepatic microsomal ACAT activity was examined in vitro in six species: rat, hamster, rabbit, African green monkey, cynomolgus monkey, and autopsy samples from normal human subjects. The animals were fed various amounts of dietary cholesterol and fat except for rats which were fed a low-fat chow diet devoid of cholesterol. As shown in **Table 1,** the specific activity varied among the species and ranged from 6.2 to 77.4 in liver and from 1.4 to 12 for intestine.

The IC_{50}s ranged from 17 to 75 nM with CP-113,818 being slightly more potent against liver ACAT activity compared to intestinal ACAT activity (29.5 ± 15 vs. 46.8 ± 29 nM, respectively; mean ± SD, $P < 0.06$, unpaired *t*-test). CP-113,818 did not inhibit lecithin:cholesterol acyltransferase or cholesteryl ester hydrolase at up to 300 μM but inhibited 50% of the pancreatic lipase activity at 300 μM, a dose approximately 14,000-fold higher than the IC_{50} for ACAT inhibition.

CP-113,818 inhibited cholesterol absorption in cholesterol-fed rats, hamsters, rabbits, cynomolgus monkeys, and African green monkeys (**Table 2**). In rats, cholesterol absorption was inhibited by 68% at 1 mg/kg which was the lowest dose tested in rats. In hamsters and

TABLE 1. In vitro inhibition of intestinal and hepatic ACAT activity by CP-113,818

Organ/Species	Specific Activity	IC_{50}[a]	Mean ± SD
	pmol/μg pro/min	*nM*	
Liver			
Rat	77.4 ± 7.4	27, 17	22
Hamster	11.0 ± 2.2	26, 18, 8	17 ± 9
Rabbit	8.8 ± 0.8	26, 24, 32	27 ± 4
African green	6.2 ± 3.2	70, 46, 24	47 ± 23
Cynomolgus	15.8 ± 8.8	46, 28, 21	32 ± 13
Intestine			
Hamster	12.0 ± 8.2	71, 24, 18	38 ± 29
Rabbit	1.4 ± 0.8	54, 15, 27	32 ± 20
African green	5.8 ± 4.6	110, 40, 40	63 ± 40
Cynomolgus	8.4, 7.0	82, 68	75
Human	12.4, 1.0	20, 39	30

[a] Data given as individual animal's IC_{50}s.

TABLE 2. Effect of CP-113,818 on cholesterol absorption inhibition in cholesterol-fed rats, hamsters, rabbits, and monkeys

Species	n	% Inhibition (vs. Controls)	Dose
			mg/kg
Rat	5	68	1.0
Hamster	16	50	0.006
Rabbit	4	50	0.01
Cynomolgus	5	40	10.0
African green	6	26	10.0

Percent inhibition values all significant at $P < 0.02$.

rabbits, cholesterol absorption was inhibited 50% compared to the untreated control animals at 9 and 10 μg/kg, respectively. In cynomolgus and African green monkeys, cholesterol absorption was inhibited 40% and 26% (mean ± SD), respectively, at 10 mg/kg. The doses of CP-113,818 selected for each species varied widely. These doses were selected based on previous experience with other ACAT inhibitors, but, in general, our series of ACAT inhibitors was more potent in hamsters > rabbits > monkeys > rats. It is unclear why potency differences exist among these species.

To determine whether longer term treatment with CP-113,818 induced intestinal ACAT activity in cholesterol-fed hamsters and rabbits, cholesterol absorption was measured repeatedly after 2–3 weeks of treatment. Intestinal ACAT activity was monitored by estimating cholesterol absorption as described previously. No changes in inhibition of cholesterol absorption were seen in either hamsters (**Fig. 2A**) or rabbits (Fig. 2B) after 2 or 3 weeks of treatment with CP-113,818, respectively.

Compared to the untreated controls, CP-113,818 prevented the accumulation of cholesterol in the liver with the highest dose of CP-113,818 given to rats, hamsters, and rabbits (150, 0.22, and 0.55 mg/kg, respectively) by 96%, 32%, and 63%, respectively (**Table 3**).

CP-113,818-treated hamsters and rats had significantly lower TPC concentrations compared to the untreated control groups (**Fig. 3**). In NZW rabbits, 3 weeks of treatment with CP-113,818 lowered TPC concentrations up to 90% compared to untreated controls (**Fig. 4**). This reduction in TPC was due primarily to decreases in β-VLDL, IDL, and LDL cholesterol, although small decreases in HDL cholesterol were seen at all doses (**Fig. 5**). There was a dose-dependent decrease in the percent of total cholesterol carried in the β-VLDL class with a concomitant increase in the percent of total cholesterol in the HDL class (**Table 4**). Although the percent of total cholesterol isolated in the LDL + IDL class remained the same with CP-113,818 treatment, there was a 90% decrease in total and LDL + IDL cholesterol. In addition, further characterization by DGUC showed a significant decrease in the IDL class (**Fig. 6**). The selective decrease in apoB-containing lipoproteins produced significant decreases in the TPC/HDL ratios in the CP-113,818-treated animals (36 ± 19, 33 ± 12, 9 ± 4*, and 5 ± 1* for the control, 0.025, 0.1, and 0.5 mg/kg per day groups, respectively; mean ± SD; *$P < 0.05$ vs. controls; unpaired *t*-test). Plasma triglyceride concentrations were unchanged with CP-113,818 treatment (data not shown).

Finally, the plasma cholesterol-lowering effect of CP-113,818 was evaluated in two species of cholesterol-fed nonhuman primates. Average TPC concentration in cynomolgus monkeys was 332 ± 59 mg/dl at baseline (mean ± SD; n = 11) compared to 271 ± 41 mg/dl after 3 weeks of treatment with CP-113,818 (**Fig. 7**). This 20% decrease in TPC was due entirely to a significant decrease in V + LDL cholesterol (296 ± 64 mg/dl vs. 215 ± 43 mg/dl; $P < 0.006$; paired *t*-test). HDL cholesterol was significantly higher after treatment (33 ± 30 mg/dl vs. 56 ± 20 mg/dl; $P < 0.002$; paired *t*-test). These lipoprotein cholesterol changes persisted after 3 additional weeks of treatment. Plasma triglyceride concentrations were unchanged with drug treatment (data not shown).

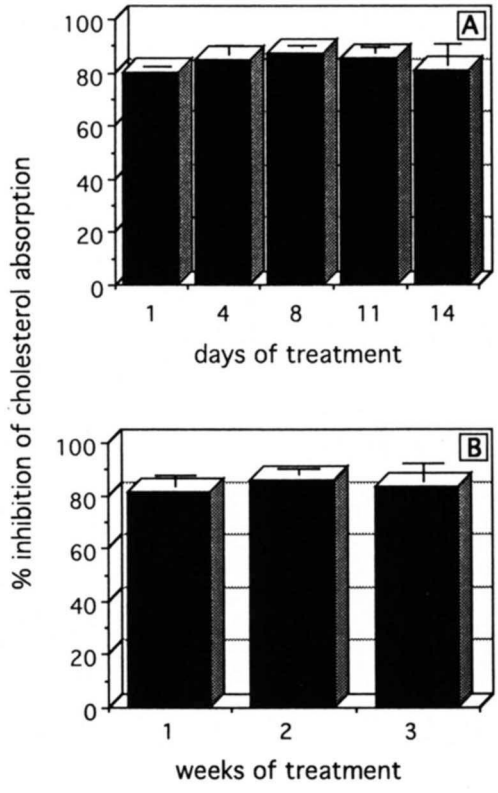

Fig. 2. The effect of CP-113,818 on cholesterol absorption in cholesterol-fed hamsters (A) and rabbits (B). Hamsters were given 0.05 mg/kg of CP-113,818 once a day by gavage for 1, 4, 8, or 11 days. Data are means ± SD; n = 3/group. Rabbits were given 0.5 mg/kg of CP-113,818 once a day orally for 1, 2, or 3 weeks. Data are means ± SD; n = 5/group.

TABLE 3. Effect of CP-113,818 on liver cholesterol mass in cholesterol-fed rats, hamsters, and rabbits

Species	Dose	Duration of Treatment	mg Cholesterol/ g liver	mg Cholesterol/ Liver
	mg/kg			
Rat	0	2 wk	47.0 ± 2.5	667 ± 37
(n = 5)	1		12.9 ± 1.9[a]	129 ± 25[a]
	30		3.2 ± 0.5[a]	30 ± 6[a]
	150		2.9 ± 0.5[a]	26 ± 6[a]
Hamster	0	2 wk	8.3 ± 0.5	55 ± 4
(n = 4)	0.03		6.6 ± 0.6	45 ± 3
	0.10		6.6 ± 0.1[b]	46 ± 2
	0.22		5.9 ± 0.2[b]	37 ± 3[b]
Rabbit	0	3 wk	7.5 ± 1.0	766 ± 129
(n = 4)	0.025		6.4 ± 1.1	552 ± 67[b]
	0.10		6.3 ± 0.4	560 ± 27[b]
	0.50		3.6 ± 0.3[b]	282 ± 29[b]

Data are given as means ± SD. Hamsters were treated with the racemic mixture of CP-113,818.
[a] $P < 0.001$; [b] $P \leq 0.02$; unpaired *t*-test.

In African green monkeys, TPC concentrations decreased approximately 60 mg/dl after 3 weeks of treatment (255 ± 86 mg/dl vs. 193 ± 49 mg/dl; $P < 0.008$; paired *t*-test). Again, the decrease in TPC was due entirely to a 34% decrease in V + LDL cholesterol (161 ± 76 vs. 107 ± 40 mg/dl; $P < 0.006$; paired *t*-test) while HDL cholesterol did not change with treatment (93 ± 20 vs. 86 ± 17 mg/dl) (Fig. 7). After 3 additional weeks of treatment, V + LDL cholesterol concentrations increased slightly but remained 22% below baseline. Plasma triglycerides concentrations were unaffected by drug treatment (data not shown).

In both species of monkeys, CP-113,818 significantly decreased TPC/HDL cholesterol ratios. This decrease was greater in cynomolgus monkeys (13.4 ± 7 vs. 5.4 ± 2; $P < 0.002$) compared to African green monkeys (2.7 ± 0.7 vs. 2.3 ± 0.5; $P < 0.02$) as treatment lowered V + LDL cholesterol and increased HDL cholesterol in cynomolgus monkeys.

In addition, there was a significant correlation between baseline V + LDL cholesterol concentrations and the change in V + LDL cholesterol in both species (**Fig. 8**). That is, the higher the initial V + LDL cholesterol concentration, the greater the decrease in V + LDL cholesterol with treatment.

Finally, after 3–4 weeks of treatment with CP-113,818, plasma concentrations of CP-113,818 were measured 16–18 h after the last dose (blood sample taken prior to morning dose). Plasma concentrations of CP-113,818 were

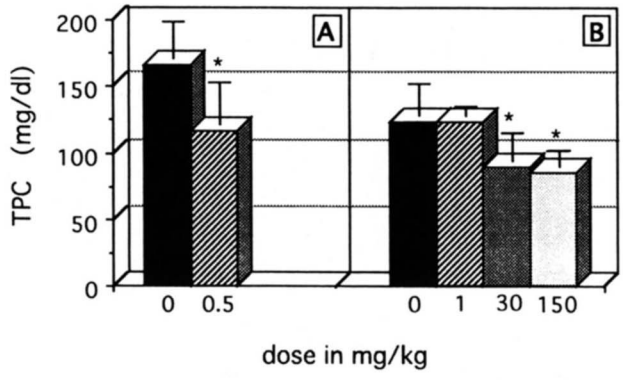

Fig. 3. The effect of 2 weeks of daily treatment with CP-113,818 on total plasma cholesterol concentrations (TPC) in cholesterol-fed hamsters (A) and rats (B). Data are means ± SD; n = 4/group for hamsters and n = 5/group for rats. • < 0.05 compared to the controls (0 dose); unpaired *t*-test.

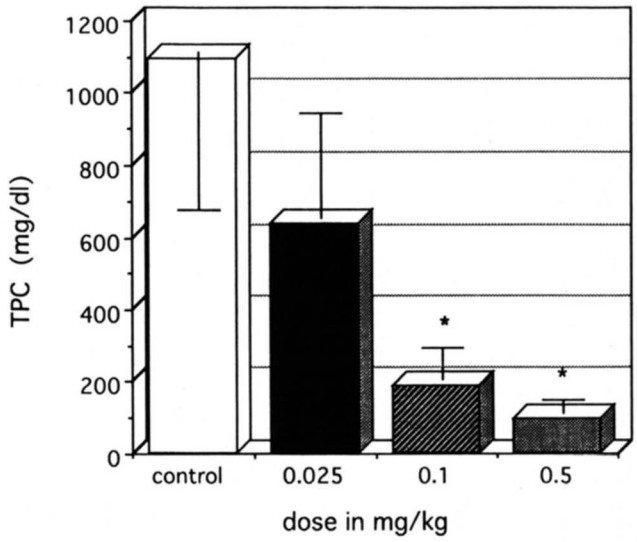

Fig. 4. Changes in total plasma cholesterol concentrations (TPC) after 3 weeks of daily treatment with CP-113,818 in cholesterol-fed rabbits. Data are means ± SD; n = 4/group; *$P < 0.05$ compared to controls; unpaired *t*-test.

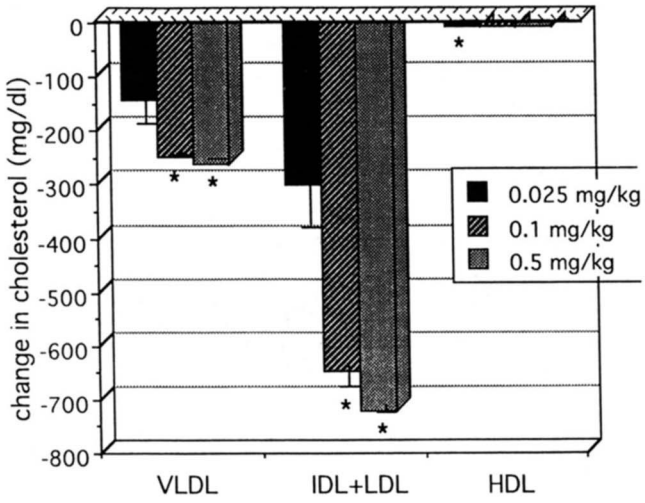

Fig. 5. Changes in lipoprotein cholesterol concentrations after 3 weeks of daily treatment with CP-113,818 in cholesterol-fed rabbits. Data are means ± SD; n = 4/group; $^*P < 0.05$ compared to controls; unpaired *t*-test.

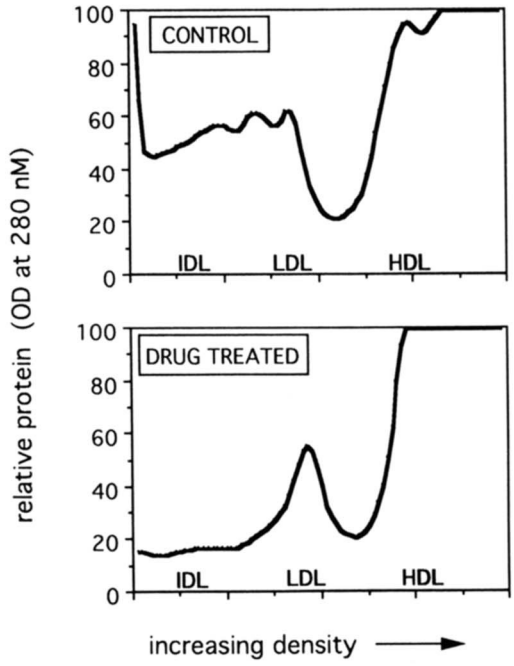

Fig. 6. Density gradient ultracentrifugation profile of d > 1.006 g/ml lipoproteins from a representative control and a drug-treated cholesterol-fed rabbit. The drug-treated rabbit received 0.5 mg/kg of CP-113,818 once a day for 3 weeks.

extremely variable and ranged between ~16 and 2035 ng/ml (365 ± 620 ng/ml; mean ± SD) for cynomolgus monkeys and ~5 and 908 ng/ml (105 ± 257 ng/ml) in African green monkeys.

In chow-fed cynomolgus monkeys, the bioavailability of CP-113,818 was 27% when administered in olive oil. In rats, the bioavailability of CP-113,818 was 28% when administered in a bolus of liquid diet (AIN #76A) (P. B. Inskeep, W. E. Ballinger, J. T. Mayne, A. G. Connolly, and K. M. Davis, unpublished results).

DISCUSSION

CP-113,818 is a potent ACAT inhibitor that has been shown to inhibit liver and/or intestinal ACAT isolated from rat, hamster, rabbit, monkeys, and human subjects. CP-113,818 also inhibited cholesterol absorption and lowered TPC in cholesterol-fed rats, hamsters, rabbits, and monkeys. Furthermore, in cholesterol-fed rabbits and

TABLE 4. Effect of CP-113,818 on the distribution of plasma cholesterol among the major lipoprotein classes in cholesterol-fed NZW rabbits

Drug Group	TPC	VLDL	IDL + LDL	HDL
mg/kg/day	*mg/dl*	%	%	%
0 (controls)	1093 ± 576	22 ± 5	74 ± 3	4 ± 3
0.025	638 ± 285	17 ± 8	79 ± 7	3 ± 1
0.10	186 ± 90	9 ± 4	78 ± 7	14 ± 6
0.50	98 ± 34	7 ± 4	74 ± 4	19 ± 3

Data given as means ± SD; n = 4 per group.

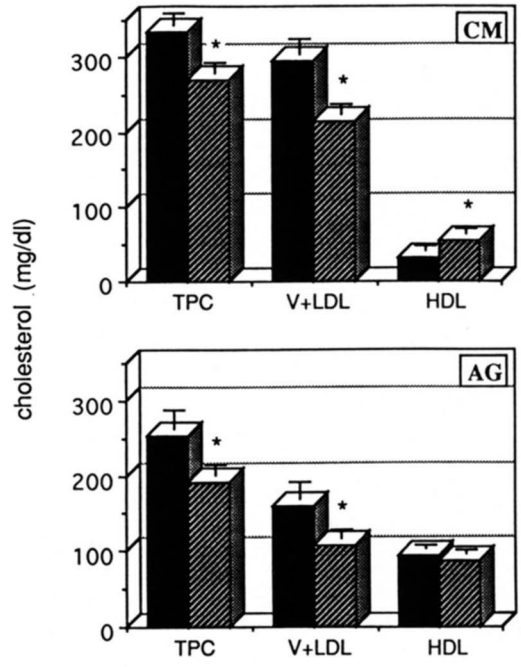

Fig. 7. The effect of CP-113,818 on total and lipoprotein cholesterol concentrations in cholesterol-fed cynomolgus macaques (CM) and African green monkeys (AG); baseline, solid bars, data prior to treatment; post 3 wks, striped bars, data after 3 weeks of CP-113,818 at either 10 or 30 mg/kg per day. Data given as means ± SEM; n = 11 for CM; n = 12 for AG; $^*P < 0.03$, paired *t*-test.

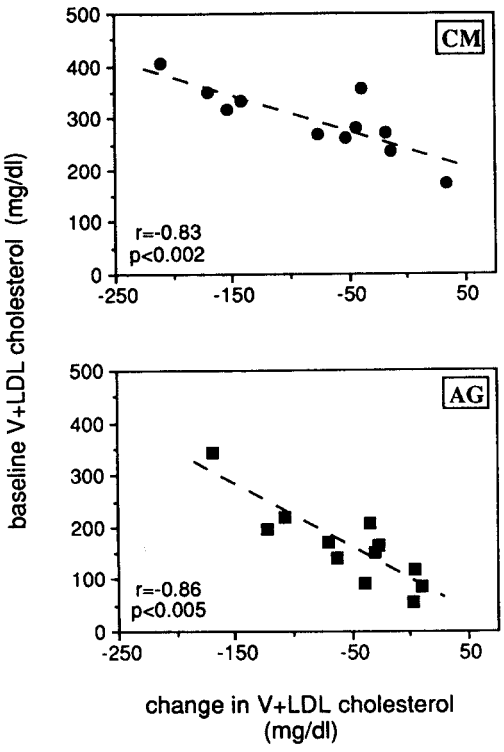

Fig. 8. The relationship between initial baseline LDL cholesterol concentrations and the change in LDL cholesterol induced after 3 weeks of treatment with either 10 or 30 mg/kg per day of CP-113,818 in CM and AG monkeys. Each symbol represents an individual animal.

monkeys, CP-113,818 selectively decreased the concentrations of apoB-containing lipoproteins and significantly improved the TPC/HDL cholesterol ratios.

The role of ACAT in cellular cholesterol metabolism has been recognized for many years. ACAT inhibitors have been shown to inhibit cholesterol absorption in cholesterol-fed rats, hamsters, and rabbits (29, 31–34). The studies presented here also show the effect of ACAT inhibition on cholesterol absorption in cholesterol-fed monkeys. However, with CP-113,818 treatment, a significant correlation between cholesterol absorption inhibition and TPC lowering was seen in cholesterol-fed hamsters and rabbits ($r = 0.89$, $P < 0.001$ and $r = 0.86$, $P = 0.0005$, respectively) which suggests that much of the plasma cholesterol lowering in these species was due to inhibiting cholesterol absorption. This correlation between plasma cholesterol lowering and cholesterol absorption inhibition was not seen in either species of nonhuman primate during treatment ($r = 0.21$ and $r = 0.23$; cynomolgus and African green monkeys, respectively). These data suggest that plasma cholesterol homeostasis may not be regulated by cholesterol absorption via ACAT inhibition to the same extent in monkeys compared to hamsters and rabbits. That is, unlike hamster and rabbit, plasma cholesterol lowering in monkeys could not be explained by the effect of CP-113,818 on cholesterol absorption alone. It seems likely that there was an additional effect of CP-113,818 on plasma cholesterol lowering by inhibiting liver ACAT activity as mean plasma levels of the compound measured 16–18 h after dosing still exceeded the IC_{50}s determined for liver microsomal ACAT activity in both species (Table 1). Consistent with this hypothesis is a study (T. P. Carr, R. L. Hamilton, Jr., and L. L. Rudel, unpublished results) in which inhibition of liver ACAT activity has been shown to decrease the secretion of lipoprotein cholesterol and apoB in perfused livers from African green monkeys.

An important aspect of this ACAT inhibitor was its selective effect on lowering apoB-containing lipoproteins in cholesterol-fed rabbits and monkeys. CP-113,818 was shown to dramatically decrease the cholesterol concentrations of the atherogenic lipoproteins β-VLDL, IDL, and LDL in cholesterol-fed rabbits. These decreases resulted in a favorable shift in the distribution of plasma cholesterol from β-VLDL to HDL and significant decreases in TPC/HDL cholesterol ratios. These results are different from the reported effects of another ACAT inhibitor (CL-277,082) that was given to cholesterol-fed rabbits at ~60 mg/kg for 4 weeks (34). This dose of CL-277,082 is approximately 120 times higher than the high dose of CP-113,818 used in the present study. With CL-277,082 treatment, VLDL cholesterol decreased ~4-fold but IDL + LDL cholesterol increased ~2.4-fold, whereas CP-113,818 treatment produced a 45-fold decrease in VLDL and an 11-fold decrease in IDL + LDL.

In cholesterol-fed African green and cynomolgus monkeys, animal models that more closely resemble human subjects in their lipoprotein profiles and responses to dietary cholesterol, CP-113,818 lowered plasma V + LDL cholesterol significantly and either did not affect HDL cholesterol (African green monkeys) or significantly increased HDL cholesterol (cynomolgus monkeys), thereby significantly reducing TPC/HDL cholesterol ratios. The magnitude of the decrease in V + LDL cholesterol was significantly correlated to the baseline V + LDL cholesterol concentrations. That is, the higher the baseline V + LDL levels, the greater the decrease produced by CP-113,818. This may be due to elevated ACAT activity levels in the more hypercholesterolemic animals. Overall, the selective decrease in apoB-containing lipoproteins with CP-113,818 treatment reduced the atherogenicity of the lipoprotein profiles in both cholesterol-fed rabbits and monkeys.

Although some ACAT inhibitors have been shown to be toxic in various animals, the various animals treated for up to 6 weeks with CP-113,818 were healthy and showed no gross abnormalities at necropsy.

As a therapeutic approach for lowering plasma cholesterol and thereby decreasing the risk of coronary heart disease, ACAT inhibition has at least two potential

advantages as a hypolipidemic agent. First, ACAT inhibition has the potential of lowering plasma cholesterol concentrations by inhibiting cholesterol absorption in the intestine as well as by decreasing lipoprotein production and secretion by the liver. Although the role of ACAT in cholesterol absorption in human subjects is unclear, it has been suggested that the high intestinal ACAT activity measured in human small intestine indicates that ACAT could play a significant role in cholesterol absorption (2). To date, only one clinical study has been published on the effect of an ACAT inhibitor (CL-277,082) on cholesterol absorption in normolipidemic human subjects (35). In this study, 750 mg/day of CL-277,082 was given to normal human subjects for 2 weeks and cholesterol absorption was measured using the dual isotope method. No significant effect was seen on cholesterol absorption. The authors offered several possible explanations for why this compound did not inhibit cholesterol absorption (e.g., too low a dose, timing of dose, etc.) but it is not clear how much of a role intestinal ACAT may play in cholesterol absorption in human subjects.

Second, ACAT inhibitors have the potential for being anti-atherogenic at the level of the artery wall, independent of the potential plasma cholesterol-lowering effects. In two recent studies in cholesterol-fed, arterial injury models, Bocan et al. (12, 13) have shown that ACAT inhibitor CI-976 prevented the progression of atherosclerosis and the accumulation of arterial cholesteryl ester in rabbits, and prevented the accumulation of arterial cholesteryl ester in Yucatan micropigs. In both animal models, arterial free cholesterol levels were also lower than that seen in the progression controls. These studies are encouraging although it will be important to validate these observations in human subjects.

In summary, the results of these studies suggest that ACAT inhibition may be a useful therapeutic approach for lowering LDL cholesterol and thereby reducing the risk of developing coronary heart disease.

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