Plasma Cholesterol Reducing Effect of FR129169, a Novel Acyl-CoA:Cholesterol Acyltransferase Inhibitor, in the Rat

Yuri Sakuma, Hiroyuki Hagihara, Kazuhiko Ohne, Akira Nagayoshi, Seitaro Mutoh*, Yoshikuni Ito, Yoshitada Notsu and Masakuni Okuhara

Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 5–2–3, Tokodai, Tsukuba, Ibaraki 300–26, Japan

Received July 17, 1995 Accepted October 16, 1995

ABSTRACT—FR129169 (FR) (*N*-(1,2-diphenylethyl)-2-octyloxyphenylacetamide) has been found to inhibit acyl-CoA:cholesterol acyltransferase (ACAT) activities in intestinal microsomes of rats and rabbits and the liver homogenate of rats with IC_{50} values of around 1.0×10^{-7} M. The inhibitory activity was 2–3 times more potent than that of CI 976 (CI). When FR in a dose of 10 mg/kg/day was administered as a dietary admixture, plasma cholesterol levels were normalized in rats fed a high cholesterol diet, but lower doses of FR had no effect. Similar results were obtained in the rats treated with CI. The ex vivo study where hepatic ACAT activity was measured after oral dosing of the two inhibitors revealed that ACAT activity was significantly reduced in rats treated with FR in a dose of 10 mg/kg/day, while CI reduced the activity at lower doses such as 0.1 and 1 mg/kg/day. Since FR was not orally absorbed, it is speculated that the inhibitory activity of FR on hepatic ACAT in the ex vivo study results from the reduction of plasma cholesterol levels. These results suggest that FR exerted cholesterol-lowering activity mainly through inhibition of intestinal ACAT activity. The significance of intestinal ACAT inhibition by FR for therapeutic treatment of hypercholesterolemia is discussed.

Keywords: Acyl-CoA:cholesterol acyltransferase, Atherosclerosis, Hypercholesterolemia, Cholesterol absorption, FR129169

Acyl-CoA:cholesterol acyltransferase (EC 2.3.1.26, ACAT) is an important enzyme in developing hypercholesterolemia and atherosclerosis. In the intestine, cholesterol is absorbed in a free form and esterified in the mucosal cell by ACAT and incorporated into chylomicrons together with triglycerides (TG), phospholipids and apolipoproteins (1). ACAT also plays an important role in the assembly of very low density lipoprotein (VLDL) in the liver (2). As excess free cholesterol becomes toxic to cells, it is first converted into esterified form by ACAT and then accumulated in vascular tissues and in macrophages in atheroma (3, 4). These facts indicate that inhibition of ACAT is a useful approach for preventing the development of atherosclerosis by reducing intestinal cholesterol absorption and inhibiting VLDL secretion from the liver, and preventing excess cholesterol deposition in arterial walls (5).

Many ACAT inhibitors have been synthesized, and their anti-hypercholesterolemic effects have been investigated in animals (6–9). Although most of them lower blood cholesterol levels in cholesterol-fed animals (10), it is still unclear whether their effects were due to inhibition of intestinal ACAT or hepatic ACAT.

To clarify this point, we have chosen two ACAT inhibitors in this study. One is CI 976 (CI) which is orally absorbable and can lower blood cholesterol levels by reducing cholesterol absorption at the intestine and by inhibiting secretion of VLDL from the liver (11). Another one is FR129169 (FR) which exhibits inhibitory activities on ACATs of various tissues in in vitro tests but is not orally absorbed. Therefore, it can be assumed that FR can not inhibit hepatic ACAT but can inhibit intestinal ACAT if it is administered orally. In this study, we characterized FR in comparison with CI and found that the two inhibitors exerted similar blood cholesterol-lowering activities in cholesterol-fed rats, although FR had much less inhibitory activity on hepatic ACAT than CI.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

Chemicals

FR129169, *N*-(1,2-diphenylethyl)-2-octyloxyphenylacetamide, and CI 976 (Warner-Lambert compound) were synthesized at Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Ibaraki (Fig. 1). [1-^{14}C]Oleoyl-CoA (2.0 MBq/μmol) was obtained from New England Nuclear (Boston, MA, USA).

In vitro potency

Preparation of enzyme source: In vitro ACAT inhibitory activity was determined in rat and rabbit intestinal microsomes and rat liver homogenate. Male, Japanese white rabbits (2.0−2.5 kg body weight, 9-weeks-old; Kitayama Labes, Kyoto) and male, Sprague-Dawley rats (180−220 g body weight, 6-weeks-old; Clea, Tokyo) were used. Rabbits were fed pelleted rabbit chow supplemented with 2% cholesterol (Oriental Yeast, Tokyo) ad libitum for 8 weeks. Rats were fed chow supplemented with 1% cholesterol and 0.5% cholic acid (Clea) ad libitum for 7 days.

After 24 hr of fasting, each animal was killed by overdosing of pentobarbital. Samples of the small intestine and liver were excised immediately. The intestinal microsome fraction was prepared by the method of Helgerud et al. (12). Two grams of rat liver from each animal was homogenized at 4℃ in 5 ml of 0.154 M potassium phosphate buffer (pH 7.4).

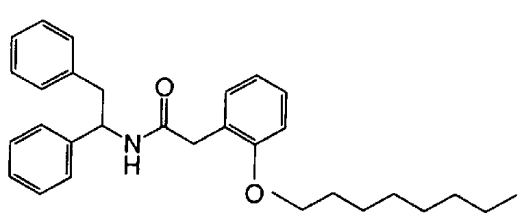
ACAT assay: The assay of ACAT activity was performed according to the method of Helgerud et al. (12) with minor modifications.

Endogenous cholesterol of the microsomal fraction or the liver homogenate and exogenous [1-^{14}C]oleoyl-CoA were used as the substrate. The incubation mixture of 150 μl of 0.154 M potassium phosphate buffer (pH 7.4) containing 1.7 nM fatty acid free BSA (Sigma, St. Louis, MO, USA) and equal molar [1-^{14}C]oleoyl-CoA was preincubated at 37℃ for 5 min before the addition of 50 μl of the enzyme source. The assay was linear up to 5 min. Therefore, a 4-min incubation time was used in the subsequent experiments. There was a linear relationship between ACAT activity and protein content of the enzyme source up to 30 μg of protein. Therefore, each assay contained 20 μg of protein (data not shown).

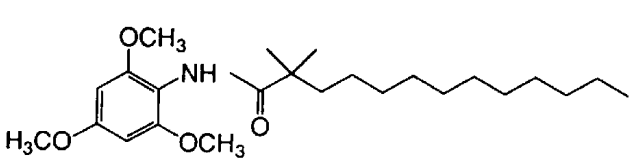
The reaction was stopped by the addition of 500 μl of chloroform/methanol (2:1, v/v). After vigorous shaking, the reaction mixture was centrifuged at 1,500×*g* for 5 min. A 250-μl aliquot of the chloroform phase was extracted and transferred with chloroform containing cholesteryl oleate as a cold carrier to a silicagel 60 plate (Merck, Darmstadt, FRG) for TLC using *n*-hexane/diethyl ether/acetic acid (73:25:2, v/v) as a solvent system. The band of cholesteryl oleate was detected by staining with iodine vapor. The fraction containing cholesteryl [1-^{14}C]-oleate was cut out and placed in counting vials. The radioactivity was measured by liquid scintillation counting with toluene scintillator in a Packard liquid scintillation counter. Esterification rates were calculated as nmol of cholesteryl [1-^{14}C]oleate formed/mg protein/min.

In vivo efficacy in cholesterol-fed rats

Male Sprague-Dawley rats (180−220 g body weight, 6-weeks-old; Clea) were used. Rats were fed the chow diet supplemented with 1% cholesterol and 0.5% cholic acid (high cholesterol diet) ad libitum. Normal control group rats were fed the standard chow. Drugs were mixed with the high-cholesterol diet at final concentrations of 0.00012%, 0.0012% and 0.012% (w/w) in order to obtain daily doses of 0.1, 1 and 10 mg/kg, respectively. The duration of feeding was 6 days. Blood samples were taken for measurement of plasma lipid parameters at 9 a.m. through the tail vein in the nonfasted state. Plasma total cholesterol, TG, free fatty acid and high density lipoprotein (HDL)-cholesterol were measured by enzymatic methods using commercial kits (Wako Pure Chemical Industries, Osaka). The rats were then fasted for 24 hr and the livers were removed after perfusion for measurement of ACAT activity. ACAT activity was assessed by using freshly prepared liver homogenate according to the method described by Gallo et al. with minor modifications (13). Two grams of liver from each animal was homogenized in 5 ml of 0.154 M potassium



FR129169



CI 976

Fig. 1. Chemical structures of FR129169 and CI 976.

phosphate buffer (pH 7.4). Each assay tube contained 10 μg protein of the homogenate and the final assay volume was 150 μl. Total cholesterol and free cholesterol in liver homogenate were measured with the commercial kits.

Drug concentration in plasma

Male Sprague-Dawley rats in the nonfasted state were dosed with 10 mg/kg or FR or CI dissolved in PEG-400, and blood samples were collected from the femoral artery, in heparin anticoagulant, at 0.5, 1, 2, 4 and 6 hr after administration.

After the blood was centrifuged, samples of plasma were added with methanol to precipitate the plasma proteins. Drugs were extracted from the supernatant into hexane, and then the hexane was evaporated. After reconstitution with methanol, samples were analyzed for drug concentration by high performance liquid chromatography.

Statistical analyses

Statistical analyses were performed by the Dunnett's multiple comparison procedure. A difference was considered to be statistically significant when the P-value was less than or equal to 0.05.

RESULTS

In vitro potency

The effects of FR and CI on ACAT activities in intestinal microsomes and liver homogenate are shown in Fig. 2. FR and CI dose-dependently inhibited all these ACAT activities from 10^{-8} to 10^{-6} M (Fig. 2: a–c). The inhibitory activity of FR was about 2–3 times more potent than that of CI. FR and CI caused 50% inhibition of cholesteryl oleate formation at 1.5×10^{-7} and 4.5×10^{-7} M (rat intestinal microsomes), 1.0×10^{-7} and 3.4×10^{-7} M (rat liver homogenate) and 7.8×10^{-8} and 1.5×10^{-7} M (rabbit intestinal microsomes), respectively.

Responses in cholesterol-fed rats

FR and CI were administered as a dietary admixture for 6 days. The amount of food consumed (about 20–21 g/body/day) and the body weight gain during this period were similar between the normal control and the cholesterol control group rats (Table 1). Effects of FR and CI on the food consumption and the body weight gain were marginal in the treated rats except for the rats treated with the highest dose of CI. The chow consumed at this dose tended to decrease (18 g/body/day), but the final body weight was not significantly different from that of the cholesterol control group.

Cholesterol feeding in the chow resulted in a significant increase in plasma total cholesterol levels about 2.6-fold of the normal control levels (the cholesterol control group: 174.7 mg/dl, the normal control group: 67.2 mg/dl) (Table 1 and Fig. 3). Plasma TG, HDL and nonesterified fatty acid (NEFA) were not affected by the cholesterol feeding. FR and CI in the highest dose (10 mg/kg/day) significantly reduced plasma total cholesterol levels to the normal control levels and also tended to decrease TG levels greatly but not significantly (46% and

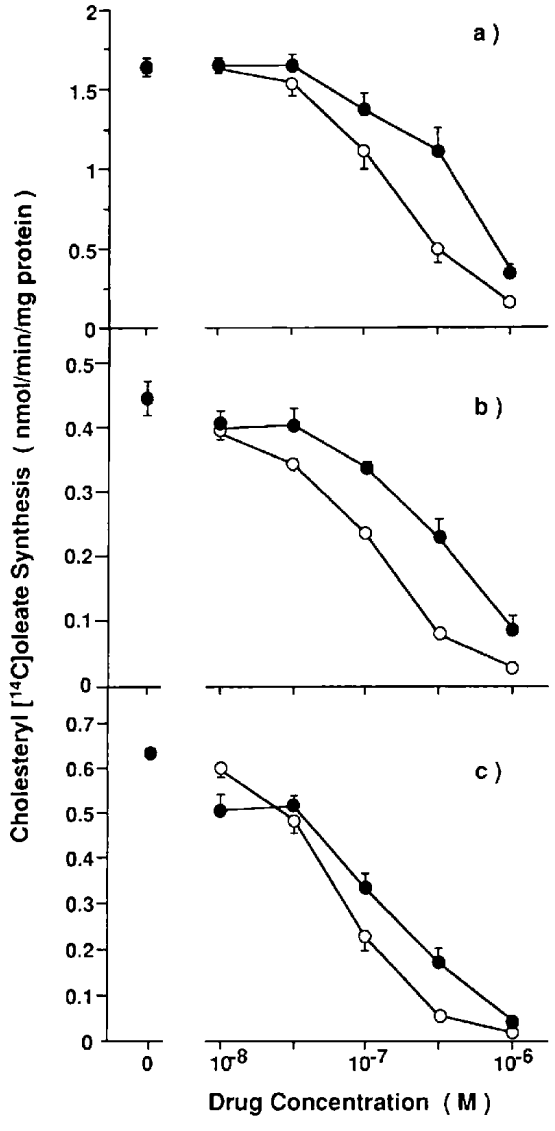


Fig. 2. Dose-response curve for the inhibitory effect of FR129169 (○) and CI 976 (●) on rat intestinal (a), rat liver (b) and rabbit intestinal (c) ACAT activity. Rat intestinal microsome, rat liver homogenate and rabbit intestinal microsomes were prepared as the enzyme sources (See methods). Endogenous cholesterol of the microsome fraction or crude liver homogenate and exogenous [1-^{14}C]oleoyl-CoA were used as the substrate. Esterification rates were calculated as nmol of [1-^{14}C]cholesteryl oleate synthesis/min/mg protein. Each point represents the mean±S.E. of values obtained in 3 experiments done in duplicate.

Table 1. Effect of ACAT inhibition on plasma lipid parameters and body weight in hypercholesterolemic rats

Treatment	Plasma cholesterol (mg/dl)	Plasma TG (mg/dl)	Plasma HDL (mg/dl)	Plasma NEFA (mEq/l)	Body weight (g)
High cholesterol diet	174.7±18.4	142.0±20.8	36.2±2.2	0.33±0.02	241.5±5.2
Normal diet	67.2± 2.6**	124.4±13.9	39.6±2.2	0.31±0.03	238.7±6.0
HC+FR 0.1 mg/kg	195.0±34.0	128.3±11.9	32.7±1.2	0.36±0.03	244.2±5.1
HC+FR 1 mg/kg	198.5±13.1	143.9± 6.5	35.8±2.1	0.45±0.05	240.4±4.6
HC+FR 10 mg/kg	56.0± 5.4**	77.0± 9.3	32.0±1.2	0.28±0.01	238.6±3.4
HC+Cl 0.1 mg/kg	179.3±22.5	119.4± 3.8	29.6±2.0	0.24±0.04	248.8±4.2
HC+Cl 1 mg/kg	171.7±27.0	160.2±36.6	34.7±2.8	0.33±0.04	244.2±3.3
HC+Cl 10 mg/kg	54.5±10.2**	101.2±22.6	26.7±3.3	0.32±0.05	224.7±4.4

HC: high cholesterol diet. Values are each the mean±S.E. for 4 rats. **P<0.01, as compared with the high cholesterol diet group.

29% reduction of the cholesterol control group levels, respectively). Although FR and CI greatly reduced total cholesterol levels, they had little effect on HDL levels.

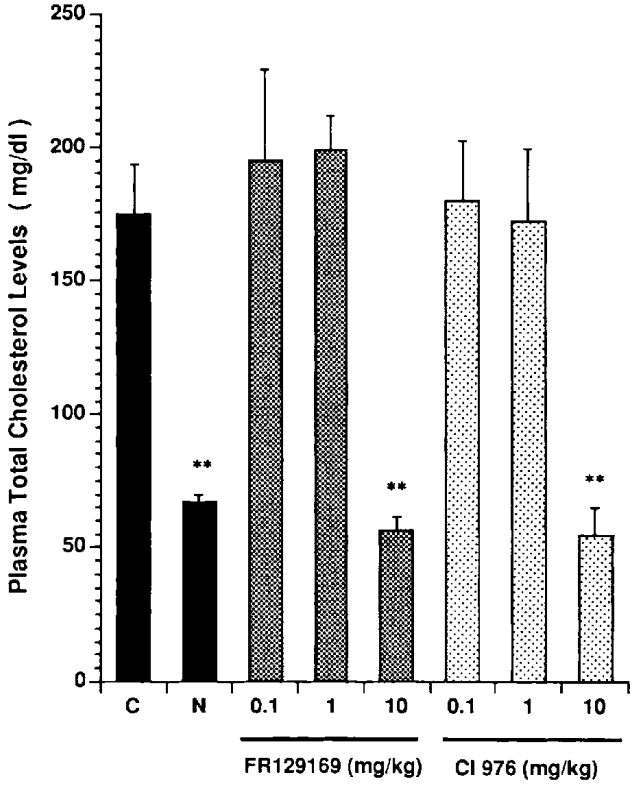


Fig. 3. Effect of FR129169 and CI 976 on plasma cholesterol levels in hypercholesterolemic rats. Rats were fed the chow diet or high cholesterol diet. Drugs were supplemented in the latter diet. The duration of feeding was 6 days. Blood samples were taken through the tail vein in the nonfasted state. Plasma total cholesterol was measured by enzymatic methods. Each data value is the mean±S.E. for 4 rats. **P<0.01, as compared with the high cholesterol fed rats. C: High cholesterol diet group, N: Normal diet group.

Cholesterol content and ACAT activity in the liver

Total cholesterol content in the liver was 19 times higher in the cholesterol control group than those of the normal control group (Fig. 4a). Although free cholesterol content was also significantly increased in the cholesterol control group, the amount of free cholesterol is very low compared to that of total cholesterol (Fig. 4b). Therefore, it is reasonable to assume that the increased amount of total cholesterol is attributable to the increase in esterified cholesterol content. Both FR and CI reduced the total cholesterol content in a dose-dependent manner with ED_{50} values of 4.8 mg/kg/day and 3.0 mg/kg/day, respectively (Fig. 4a).

Hepatic cholesteryl oleate synthesis which reflects ACAT activity was 54% higher in the cholesterol control group than in the normal control group (5.35 and 3.48 nmol/mg protein/min, respectively) (Fig. 5). FR decreased ACAT activity significantly only at a dose of 10 mg/kg/day, whereas CI decreased the activity from a dose of 0.1 mg/kg/day. Since hepatic ACAT activity is affected by plasma cholesterol levels, it is interesting to note that CI reduced hepatic ACAT activity at a dose where it had no effect on plasma cholesterol levels.

Drug concentration in plasma

Plasma concentrations of FR and CI were measured during 6 hr after oral dosing of 10 mg/kg of the each compound. The concentration of FR was too low to be detected in plasma (the detectable minimum concentration was 0.05 μg/ml), but CI could be detected and the maximum concentration (C_{max}), the time required to reach the C_{max} (T_{max}) and the area under the concentration curve (AUC, 0–6 hr) were 0.199±0.144 μg/ml, 1.7±0.6 hr and 0.527±0.344 μg/ml, respectively (n=3).

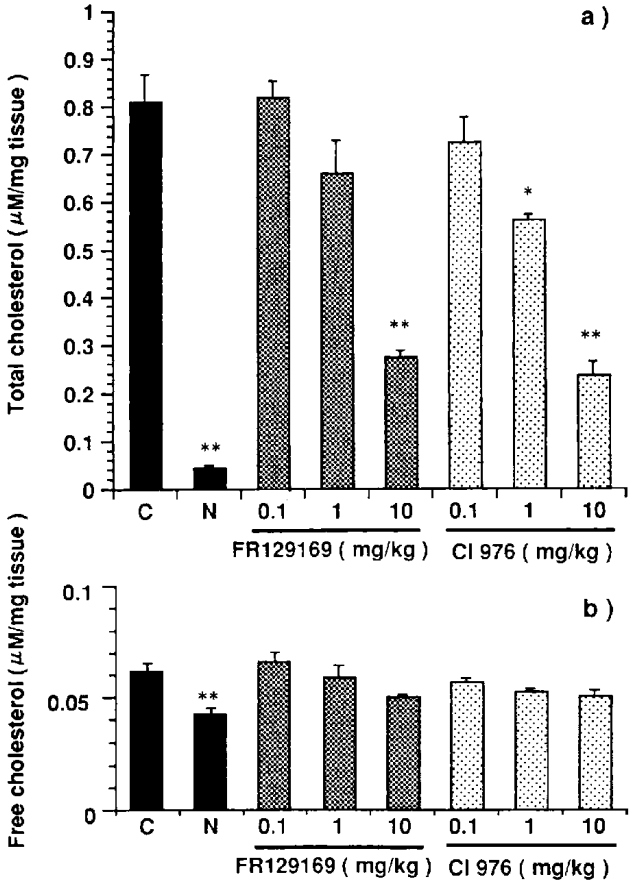


Fig. 4. Effect of FR129169 and CI 976 on liver total cholesterol (a) and free cholesterol (b) in hypercholesterolemic rats. Rats were fed the chow diet or high cholesterol diet. Drugs were supplemented in the latter diet. The duration of feeding was 6 days, and then the animals were fasted for 24 hr. The livers were removed after perfusion. Total cholesterol and free cholesterol in liver homogenate were measured by enzymatic methods. Data values are each the mean±S.E. for 4 rats. *$P<0.05$ and **$P<0.01$, as compared with the high cholesterol fed rats. C: High cholesterol diet group, N: Normal diet group.

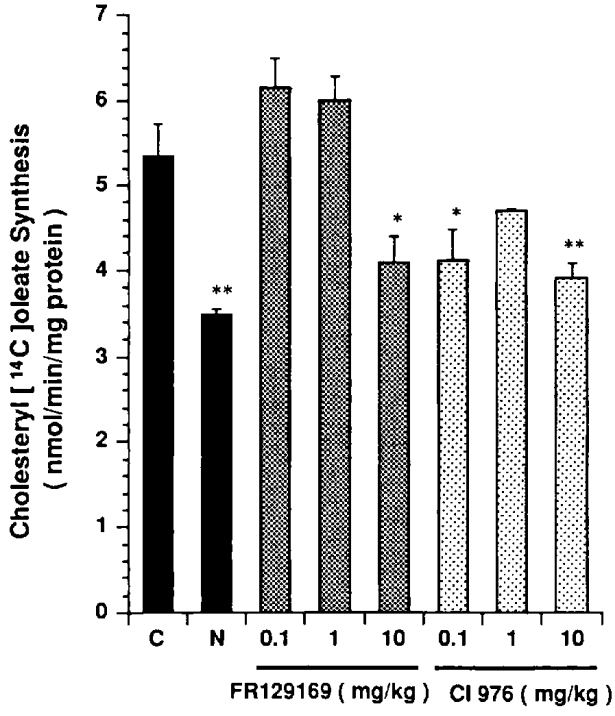


Fig. 5. Effect of FR129169 and CI 976 on liver ACAT activity. Rats were fed the chow diet or high cholesterol diet. Drugs were supplemented in the latter diet. The duration of feeding was 6 days, and then the animals were fasted for 24 hr. The livers were removed after perfusion. ACAT activity was assessed by using freshly prepared liver homogenate. Esterification rates were calculated as nmol of [1-^{14}C]oleate synthesis/min/mg protein. Data values are each the mean±S.E. for 4 rats. *$P<0.05$ and **$P<0.01$, as compared with the high cholesterol fed rats. C: High cholesterol diet group, N: Normal diet group.

DISCUSSION

The in vitro study using intestinal and hepatic ACATs revealed that FR was a more potent inhibitor than CI. However, FR had much less inhibitory action on hepatic ACAT activity in the ex vivo study. This contradiction may be due to the extremely low absorbability of FR when it is orally administered. Actually, FR could not be detected in the plasma after oral dosing of 10 mg/kg, whereas CI was well-absorbed under the same condition, suggesting that the concentration of FR in the liver might be negligible. If so, how could FR inhibit hepatic ACAT activity in the ex vivo study? When interpreting the results obtained in the ex vivo study, it is necessary to note that hepatic ACAT activity is dependent on blood cholesterol levels and that the dose in which it apparently reduced hepatic ACAT activity was the dose in which it lowered plasma cholesterol levels. Therefore, it is conceivable that the inhibitory action of FR on hepatic ACAT activity in the ex vivo study was not due to a direct inhibition of hepatic ACAT activity but due to a reduction of plasma cholesterol levels. In this context, it is interesting to note that CI inhibited hepatic ACAT in doses (0.1 and 1 mg/kg/day) lower than that needed for lowering plasma cholesterol levels (10 mg/kg/day). CI probably inhibited hepatic ACAT directly in such lower doses. The present observations lead us to speculate that FR can not inhibit hepatic ACAT activity but can inhibit intestinal ACAT activity when orally administered.

Although FR and CI had different pharmacological profiles as described above, the plasma cholesterol-lowering effects of these two compounds were seen at the same dose (10 mg/kg/day). The inhibition of hepatic ACAT might not contribute so much to the reduction of plasma cholesterol levels because CI inhibited hepatic ACAT at doses of 0.1 and 1 mg/kg/day, although the

cholesterol-lowering effect was seen only at a dose of 10 mg/kg/day. It is well-known that free cholesterol from the diet is esterified in the mucosal cell by ACAT to cholesterol esters. These esters are then incorporated into chylomicrons, which are secreted into the lymph. Therefore, inhibition of intestinal ACAT leads to the reduction of the cholesterol absorption. The importance of ACAT in the liver is less clear compared to its documented importance in the intestine. Although further studies are needed, our results suggest that inhibition of intestinal ACAT is more responsible for the cholesterol-lowering effects of ACAT inhibitors than inhibition of hepatic ACAT.

Another interesting finding in this study is that FR at a dose of 10 mg/kg also reduced plasma TG levels to about one half that of the non-treated group, although the effect was not statistically significant. The reduction of blood TG levels by an ACAT inhibitor, U-73482, in rats was also previously reported (14). TG is incorporated into chyromicrons together with cholesterol esters, phospholipids and apolipoproteins and then secreted into the blood. Esterification of cholesterol is critical for assembly of chyromicrons in the intestine (15–18). Therefore, inhibition of cholesterol ester formation by FR might interfere with the assembly of chyromicrons, possibly resulting in the reduction of plasma TG levels as well cholesterol levels.

Hypercholesterolemia is frequently associated with diabetes mellitus. One of the causes for hypercholesterolemia in the diabetic state is thought to be an increase in cholesterol absorption from the intestine. This speculation is supported by the facts that ACAT activity in the intestine is increased in both the insulin-dependent diabetic model (streptozotocin-induced diabetic rats) and non-insulin-dependent diabetic model (Wistar-fatty rat) (19–22). In addition, intestinal hyperplasia occurred in insulin-dependent diabetic patients with poor blood glucose control, and the increases in sterol absorption in these patients were well-correlated with the hyperplasia (23). These facts suggest that suppression of cholesterol absorption in the intestine by ACAT inhibition may be an effective treatment of hypercholesterolemia in diabetic patients. Recently, bile acid-binding resins such as cholestyramine and cholestipol have become available for the treatment of hypercholesterolemia. They inhibit intestinal absorption of cholesterol by interrupting enterohepatic circulation of bile acids. However, these drugs tend to raise plasma TG levels, which may not be a good pharmacological profile for the treatment of non-insulin-dependent diabetes mellitus patients with hypertriglyceridemia (24). On the other hand, FR reduced plasma TG levels and could be useful for the treatment of hyperlipidemia in diabetic patients.

In summary, FR lowered plasma cholesterol levels in cholesterol-fed rats as well as CI. The ex vivo studies revealed that FR was much less potent in inhibiting hepatic ACAT activity than CI, although the in vitro study showed that FR inhibited intestinal and hepatic ACAT activities more potently than CI. Since FR was not orally absorbed, it is possible that its cholesterol-lowering action is not due to the inhibition of hepatic ACAT but due to the inhibition of intestinal ACAT. FR also reduced plasma TG levels and had no effect on HDL levels, which are preferable profiles as a lipid lowering drug. These results suggest the usefulness of ACAT inhibition at the intestine in the treatment of hypercholesterolemia.

REFERENCES

1 Fielding CT, Renston JP and Fielding PE: Metabolism of cholesterol enriched chylomicrons. J Lipid Res **19**, 705–711 (1978)
2 Erickson SK and Shrewsbury MA: Rat liver acyl-coenzyme A:cholesterol acyl transferase. J Lipid Res **21**, 930–941 (1980)
3 Gillies PJ and Rathgeb KA: Regulation of Acyl-CoA:cholesterol acytransferase activity in normals atherosclerotic rabbit aortas. Exp Mol Pathol **44**, 329–339 (1986)
4 Tabas I and Bcykow GC: Protein synthesis inhibition in mouse peritoneal macrophages results in increased acyl-coenzyme A:cholesterol acyltransferase activity and cholesterol ester accumulation in the presence of native low density lipoprotein. J Biol Chem **262**, 12175–12181 (1987)
5 Suckling KE and Stange EF: Role of acyl-CoA:cholesterol acytransferase in cellular cholesterol metabolism. J Lipid Res **26**, 647–671 (1985)
6 Krause BR, Black A, Bousley R, Essenburg A, Cornicelli J, Holmes A, Homan R, Kieft K, Sekerke C and Shaw HMK: Divergent pharmacologic activities of PD132301-2 and CL277092, urea inhibitor of acyl-CoA:cholesterol acyltransferase. J Pharmacol Exp Ther **262**, 734–743 (1993)
7 Trivedi BK, Holmes A, Stoeber TL, Essenburg AD, Stanfield RL and Krause BR: Inhibitors of acyl-CoA:cholesterol acyltransferase. 4. A novel series of urea ACAT inhibitor as potential hypocholesterolemic agents. J Med Chem **29**, 3300–3307 (1993)
8 Bocan TM, Mueller SB, Uhlendorf PD, Newton RS and Krause BR: Comparison of CI976, on ACAT inhibitor, and selected lipid lowering agents antiatherosclepotic activity in iliac-fomeral and thoracic aortic lesions. A chemical, morphological, and morphometric evaluation. Arterioscler Thromb **11**, 1830–1843 (1991)
9 Tanaka H, Ohtsuka I, Kogushi M, Kimura T, Fujimori T, Saeki T, Hayashi K, Kobayashi H, Yamada T, Hiyoshi H and Saito I: Effect of the acyl-CoA:cholesterol acyltransferase inhibitor, E5324, on experimental atherosclerosis in rabbits. Atherosclerosis **107**, 187–201 (1994)
10 Heider JG, Fears R, Levy RI, Shepherd J and Packard JR: Agents which inhibit cholesterol esterification in intestine and their potential value in the treatment of hypercholesterolemia. *In* Pharmacological Control of Hyperlipidemia, Edited by Fears R, pp 423–438, JR Prous Science Publishers, Barcelona (1986)

11 Krause BR, Anderson M, Bisgaier CL, Bocan T, Bouslew R, DeHart P, Essenburg A, Hamelehle K, Homan R, Kieft K, McNally W, Stanfield R and Newton RS: In vivo evidence that the lipid-regulating activity of the ACAT inhibitor CI-976 in rats is due to inhibition of both intestinal and liver ACAT. J Lipid Res **34**, 279–294 (1993)

12 Heigerud PK, Saarem and Norum KR: AcylCoA:cholesterol acyltransferase in human small intestine, its activity and some properties of the enzymatic reaction. J Lipid Res **22**, 271–277 (1981)

13 Gallo LL, Wadsworth JA and Vahouny GV: Normal cholesterol absorption in rats deficient in intestinal acylcoenzyme A:cholesterol acyltransferase activity. J Lipid Res **28**, 381–387 (1987)

14 Bell FP, Gammill RB and St John LC: U-73482: A novel ACAT inhibitor that elevates HDL-cholesterol, lower plasma triglyceride and facilitates hepatic cholesterol mobilization in the rat. Atherosclerosis **92**, 115–122 (1992)

15 Kam NTP, Albright E, Mathur SN and Field FJ: Inhibition of acylcoenzyme A:cholesterol acyltransferase activity in CaCo-2 cells results in intracellular triglyseride accumulation. J Lipid Res **30**, 371–377 (1989)

16 Bennett CS and Tercyak AM: Reduced cholesterol transmucosal transport in rats with inhibited mucosal acyl CoA:cholesterol acyltransferase and normal pancreatic function. J Lipid Res **25**, 148–159 (1984)

17 Tanaka M, Jingami H, Otani H, Cho M, Ueda Y, Arai H, Nagano Y, Doi T, Yokode M and Kita T: Regulation of apolipoprotein B production and secretion in response to the change of intracellular cholesterol ester contents in rabbit hepatocytes. J Biol Chem **268**, 12713–12718 (1993)

18 Dashti N: The effect of low density lipoproteins, cholesterol, and 25-hydroxycholesterol on apolipoprotein B gene expression in HepG2 cells. J Biol Chem **267**, 7160–7169 (1992)

19 Jiao S, Matsuzawa Y, Matsubara K, Kudo M, Tokunaga K, Odaka H, Ikeda H, Matsuo T and Tarui S: Abnormalities of plasma lipoproteins in a new genetically obese rat with non-insulin-dependent diabetes mellitus (Wistar fatty rats). Int J Obesity **15**, 487–495 (1991)

20 Jiao S, Matsuzawa Y, Matsubara K, Kihara S, Nakamura T, Tokunaga K, Kubo M and Tarui S: Increased activity of intestinal acyl-CoA:cholesterol acyltransferase in rats with streptozosin-induced diabetes and restoration by insulin supplementation. Diabetes **37**, 342–346 (1988)

21 Yang LY, Kuksis A and Steiner G: Comparison of the effect of hyperinsulinemia acyl-CoA:cholesterol acyltransferase activity in the liver and intestine of the rat. Atherosclerosis **107**, 25–34 (1994)

22 Maecher P, Wollheim CB, Bentzen CL and Niesor E: Importance of exogenous cholesterol in diabetic rats: effect of treatment with insulin or with an acyl-CoA:cholesterol acytransferase inhibitor. Ann Nutr Metab **37**, 199–209 (1993)

23 Kojima H, Hidaka H, Matsumura K, Konaka K, Kawabata T and Shigeta Y: Clinical study of exogenous sterol absorption and post-heparin plasma diamine oxidase (DAO) activity. J Jpn Atheroscler Soc **22**, 85 (1994) (Abstr in English)

24 Garg A: Lipid-Lowering therapy and macrovascular disease in diabetes mellitus. Diabetes **41**, Supp **2**, 111 (1992)