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DEMONSTRATION OF A DIRECT EFFECT ON HEPATIC ACYL CoA:CHOLESTEROL ACYL TRANSFERASE (ACAT) ACTIVITY BY AN ORALLY ADMINISTERED ENZYME INHIBITOR IN THE HAMSTER

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Abstract—Orally active inhibitors of acyl CoA:cholesterol acyl transferase (ACAT), such as Lederle CL277082 (LE), are known to reduce plasma and hepatic cholesteryl ester levels, although the mechanisms are not well understood. Several groups have reported the inhibition of cholesterol absorption upon oral ACAT inhibitor administration. In this study, we used 7-day dietary and drug treatments of hamsters to examine the possible effects of LE on hepatic ACAT. ACAT assays were performed using liver homogenates in the absence and presence of a saturating level of exogenously added cholesterol. LE (100 mg/kg/day) treatment of chow or 0.5% cholesterol-fed animals caused reductions in ACAT activity without additional cholesterol as compared with non-treated animals. When a saturating level of cholesterol was added to the assays, reductions in ACAT activity upon LE treatment of chow- or cholesterol-fed animals were also observed. Treatment of cholesterol-fed animals with cholestyramine in the diet reduced ACAT activity in the absence of added cholesterol. However, ACAT activities similar to those of non-treated animals were observed at a saturating level of cholesterol. This latter effect demonstrates that inhibition of cholesterol absorption reduces cholesterol delivery to the liver but does not reduce cholesterol esterifying capacity since cholestyramine is not absorbed and has no direct effect on the liver. The decreased ACAT activity in homogenates from LE-treated animals could also be mimicked in a dose-dependent manner by the addition of exogenous LE to liver homogenates from non-treated animals. These results indicate that hepatic ACAT activity is regulated by the availability of free cholesterol, and that orally administered LE has a direct effect on hepatic ACAT activity in the liver. In addition, the data are consistent with LE activity in the liver as being responsible, in part, for the reduced hepatic and plasma cholesteryl esters in treated animals.

Key words: liver; acyl CoA:cholesterol acyl transferase; enzyme inhibition; hamsters; cholesterol; hypocholesterolemic agents

The importance of ACAT† in cholesterol metabolism has led to a number of studies describing the function of this enzyme in a variety of tissues [1, 2]. The enzyme is located in the endoplasmic reticulum and has been shown to respond to increased cellular cholesterol by catalyzing the formation of cholesteryl esters. Once the esters have been synthesized, they may associate with newly forming lipoproteins. In the intestine and liver, cholesteryl esters become associated with chylomicrons and very low density lipoproteins (VLDL), respectively. In recent studies, Carr *et al.* [3], using perfused livers from cholesterol-fed primates, demonstrated that hepatic ACAT activity increases the atherogenicity of plasma low density lipoprotein (LDL) by increasing cholesteryl oleate content, suggesting the potential importance of this enzyme in the atherogenic process. Cholesteryl esters may also accumulate within tissues as lipid droplets, which are commonly seen in foam cells of the atherosclerotic lesion.

Due to the potential role of ACAT in the accumulation of cholesteryl esters in the arterial wall and plasma lipoproteins, several efforts to design specific inhibitors of ACAT as therapy for hypercholesterolemia and atherosclerosis have been reported [4–6]. Oral administration of such inhibitors has been shown to inhibit the absorption of cholesterol in the intestine, causing increased fecal excretion of sterols. Since absorbed cholesterol is delivered to the liver rapidly and efficiently via the chylomicron/chylomicron remnant pathway, ACAT inhibition results in decreased hepatic accumulation of cholesterol. In several studies, oral administration of ACAT inhibitors has also been shown to reduce serum cholesterol levels, leading to the conclusion that ACAT activity has been decreased [4, 7]. The mechanism for the reduced serum cholesterol levels has not been elucidated but may be due to decreased hepatic cholesterol from which to synthesize cholesteryl ester [8] or potentially through a direct inhibition of hepatic ACAT.

The ACAT protein has not been purified and assessments of the molecular mechanism of action

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† Abbreviations: ACAT, acyl CoA:cholesterol acyl transferase (EC 2.3.1.26); and LE, Lederle ACAT inhibitor CL277082 (1-heptyl-3-(2,4-difluorophenyl)-1-[4-(2,2-dimethyl)-propylbenzyl]urea).

have not been performed in well characterized systems. Using crude membrane cell extracts and membrane fractions, several laboratories have reported enzyme activation by the presence of oxygenated sterols or agents that promote protein phosphorylation. Enzyme activity has also been demonstrated to be increased in cultured cells incubated in the presence of lipoproteins [1] or in membrane fractions [9–11] in which exogenous cholesterol has been introduced from acetone or detergent micelles. In the present study, we performed kinetic experiments to ascertain whether the apparent reduced ACAT activity is due to reduced delivery of cholesterol to the liver from the intestine, or whether orally administered compounds directly cause ACAT inhibition in the liver. The experiments employed oral administration of CL277082 (LE), as a well studied ACAT inhibitor [5, 7], in the hamster. LE is absorbed and incorporated into bile via the liver [12]; however, direct hepatic effects of the compound have not been reported. Cholesterol-fed hamsters have been chosen due to similarities with respect to human lipid metabolism and effects of LE on cholesterol absorption and serum cholesterol levels [13]. For comparative purposes, we also studied the effects of the bile acid sequestrant cholestyramine, since this agent inhibits cholesterol absorption and subsequently reduces the amount of cholesterol delivered to the liver. Since the sequestrant is not absorbed, metabolic effects observed at the liver are not complicated by the potential for a direct drug action at this site. The results of this study indicate that the ACAT inhibitor CL277082 was absorbed and has the potential to inhibit hepatic ACAT activity *in vivo*. The experimental paradigm utilized in this study may be used to determine the role of hepatic ACAT inhibition as part of the mechanism of action of other hypolipidemic agents that have the potential to reduce cholesterol esterification.

MATERIALS AND METHODS

Materials. All diets were prepared by Research Diets, Inc., New Brunswick, NJ. The corn oil, cholestyramine, cholesterol kits and other reagent grade chemicals were obtained from the Sigma Chemical Co., St. Louis, MO. Lipid standards were obtained from Nu-Check Prep, Inc., Elysian, MN. [9,10-^{3}H]Oleic acid (5 Ci/mmol) was purchased from Amersham, Arlington Heights, IL; HPLC grade solvents were obtained from Fisher, Springfield, NJ.

Animal treatment. Male Golden Syrian hamsters (125–150 g) were divided into groups of four animals. As indicated, some of the animals were maintained on a cholesterol-free standard rodent chow diet. Other animals were fed the chow diet containing 0.5% cholesterol for a period of 7 days. LE was administered once daily at the indicated dosage levels in 0.2 mL corn oil by oral gavage. Animals receiving bile acid sequestrant were placed on a chow diet supplemented with both 0.5% cholesterol and 2.5% cholestyramine. Animals not receiving the CL277082 were gavaged with 0.2 mL corn oil to account for any compound vehicle effects. Plasma and hepatic samples were obtained after a 7-day treatment period. All animals were treated and cared for according to NIH guidelines for humane treatment of laboratory animals [14] and the Animal Welfare Act in a program accredited by the American Association for Accreditation of Laboratory Animal Care.

Preparation of [^{3}H]oleoyl CoA. Radiolabeled oleoyl CoA was prepared according to the method described by Bishop and Hajra [15]. Briefly, 2 mCi of [^{3}H]oleic acid was reduced to the acyl chloride and reacted with coenzyme A, resulting in a 40% yield of a preparation that had a specific activity of 1.8 Ci/mmol. The preparation was judged to be greater than 95% pure based on cellulose thin-layer chromatography using a solvent system that contained *n*-butanol : acetic acid : water (5 : 2 : 3, by vol.). This material was divided into small aliquots and frozen in tubes containing an argon atmosphere at −70° until needed.

Assay for ACAT activity. Assays for ACAT activity were performed using the incubation conditions described by Tabas *et al.* [16] with several modifications. In the indicated assays, either liver homogenates or isolated microsomes were used as an enzyme source. The homogenates were prepared daily from frozen liver tissue. In separate experiments, similar results were obtained using either fresh or frozen tissue. The homogenates were prepared using 0.25 g of liver tissue and 1 mL of buffer containing 0.25 M sucrose, 1 mM EDTA, and 10 mM Tris (pH 7.4) and homogenized with an Omni 1000 hand-held homogenizer (Omni International, Waterbury, CT). Unless indicated otherwise in the text, incubations were prepared containing 90 μM bovine serum albumin (essentially fatty acid free), 12.5 μg microsomal protein or 50 μg homogenate protein in a buffer containing 0.1 M potassium phosphate and 2 mM dithiothreitol (pH 7.4) in a total volume of 50 μL. Exogenously added LE was introduced into the incubations where indicated from 1 μL of concentrated stock solutions in dimethyl sulfoxide. Exogenous cholesterol was added to incubations from acetone stock solutions in a 2-μL aliquot. After preincubation at 37° for 15 min, [^{3}H]-oleoyl CoA (10 μM final concentration, 1 μCi per tube) was added, and incubations were continued at 37° for 15 min. Assays were terminated by the addition of a 15-μL aliquot of each incubation directly to silica gel G thin-layer plates. The thin-layer plates were allowed to dry for several minutes and then developed using a solvent containing petroleum ether : diethyl ether : acetic acid (90 : 10 : 1, by vol.). Direct application of the thin-layer plates with incubation mixtures is rapid, reproducible and overcomes formation of radioactive reaction products previously documented in ACAT reactions [17]. Regions corresponding to the migration position of standard cholesteryl esters were scraped, and the radioactivity was quantified by scintillation spectrometry.

Standard laboratory procedures. Non-fasted plasma samples were obtained from each hamster, and total serum cholesterol was determined enzymatically [18]. Rat liver microsomes were prepared by differential centrifugation according to a method described previously [19] and stored at

Table 1. Effects of diet and drug treatment on hamster plasma and liver cholesterol content

Treatment group	Total plasma cholesterol (mg/dL)	Liver cholesteryl ester (mg/g liver)	Liver free cholesterol (mg/g liver)
Chow-fed	116 ± 4	1.83 ± 0.18	1.25 ± 0.09
Chow-fed + LE (100 mg/kg/day)	82 ± 9*	1.70 ± 0.04	1.37 ± 0.05
Cholesterol-fed	276 ± 10*	28.40 ± 2.61*	1.61 ± 0.19*
Cholesterol-fed + LE (25 mg/kg/day)	220 ± 11†	11.06 ± 1.23†	1.32 ± 0.22
Cholesterol-fed + LE (100 mg/kg/day)	159 ± 32†	1.84 ± 0.57†	1.47 ± 0.27
Cholesterol-fed + CY (2.5% of diet)	89 ± 5†	0.42 ± 0.04†	1.04 ± 0.12†

Animals (N = 4–6/group) were treated for 7 days with the indicated diet and drug treatments. CL277082 (LE) was administered once daily by oral gavage in 0.2 mL corn oil, and cholestyramine (CY) was mixed in the diet. Data are shown as the mean ± SEM for each group.

* $P < 0.05$ compared with chow-fed control.

† $P < 0.05$ compared with cholesterol-fed control.

−80° in small aliquots until needed. Protein assays were performed according to the method of Lowry *et al.* [20] using bovine serum albumin as a reference.

Analysis of liver neutral lipid fractions. Samples of liver tissue or liver microsomes were extracted according to the procedure of Folch *et al.* [21]. Lipid extracts were dried under nitrogen in HPLC sample vials, resuspended in hexane, and injected onto a Zorbax Sil (4.6 × 25 cm) silica column. Chromatography was performed essentially as described by Hamilton and Comai [22] using an isocratic mobile phase containing 98.8% hexane and 1.2% isopropanol and a flow rate of 2 mL/min. Lipids were detected by absorbance at 206 nm and quantified by computer integration (System Gold, Beckman) of elution profiles. Elution times for cholesteryl ester, triglyceride, and free cholesterol were 1.45, 1.72, and 9.10 min, respectively. Cholesterol concentrations were determined by the use of a response factor made from a standard curve using known amounts of cholesterol. The cholesteryl ester content of the liver-derived samples was based on a response factor of the weighted average of cholesteryl esters present in the livers from hamsters fed the cholesterol-containing diet for 7 days.

RESULTS

The effects of dietary cholesterol and drug treatment on the serum cholesterol levels and hepatic free and esterified cholesterol levels are shown in Table 1. Feeding of a diet containing 0.5% cholesterol for 7 days caused a 2.4-fold increase in plasma cholesterol levels as compared with levels in animals maintained on a chow diet. An oral gavage of the ACAT inhibitor CL277082 (LE) once daily reduced the serum cholesterol levels in a dose-dependent manner in the cholesterol-fed animals, although serum cholesterol levels similar to those of chow-fed animals were not observed even at a dose of 100 mg/kg/day. Treatment of chow-fed animals with LE caused a 29% reduction in serum cholesterol levels. Inclusion of cholestyramine in the cholesterol-containing diet effectively maintained serum cholesterol levels below normal levels despite the dietary cholesterol load. More striking effects of cholesterol feeding were observed in the accumulation of liver cholesteryl esters. At the end of the 7-day treatment period, over a 15-fold increase in hepatic cholesteryl esters was found as compared with levels in the chow-fed group, and the overall trend for drug action on liver cholesteryl ester accumulation was similar to that observed for serum cholesterol levels. In comparison with the large changes observed with cholesteryl esters, free cholesterol levels remained relatively constant although some increase was noted upon cholesterol feeding. No differences in either the weight of the animals or in the weight of the livers from any of these animals were observed after the 7-day treatment period. The data indicate that the liver is very responsive to dietary cholesterol and responds with a large accumulation of cholesteryl esters, which can be attenuated by oral administration of LE.

To account for the large changes in cholesteryl ester accumulation in the liver, we sought to examine ACAT activity in detail. For this purpose, modifications of standard microsomal ACAT assays were made to more fully characterize the activity of this enzyme in liver samples. In addition, the assay employed for these studies does not include an organic extraction of each incubation and avoids excess manipulation. To avoid potential fractionation of cholesterol substrate or ACAT inhibitor away from the enzyme during the preparation of microsomes, the assay employed for these studies utilized whole tissue homogenates. The kinetic parameters for the assay, comparing the use of microsomes and homogenates from cholesterol-fed animals, are shown in Fig. 1. Although the amounts of cholesteryl ester formed were different for microsomes and homogenates, the curves demonstrated similar dose–response relationships using either enzyme source with respect to incubation time and acyl CoA concentration and indicated that the assay is essentially linear for both homogenates and microsomes with respect to protein. From these results, standard conditions with homogenates in subsequent experiments employed 50 μg protein, 10 μM oleoyl CoA, and a 15-min incubation period.

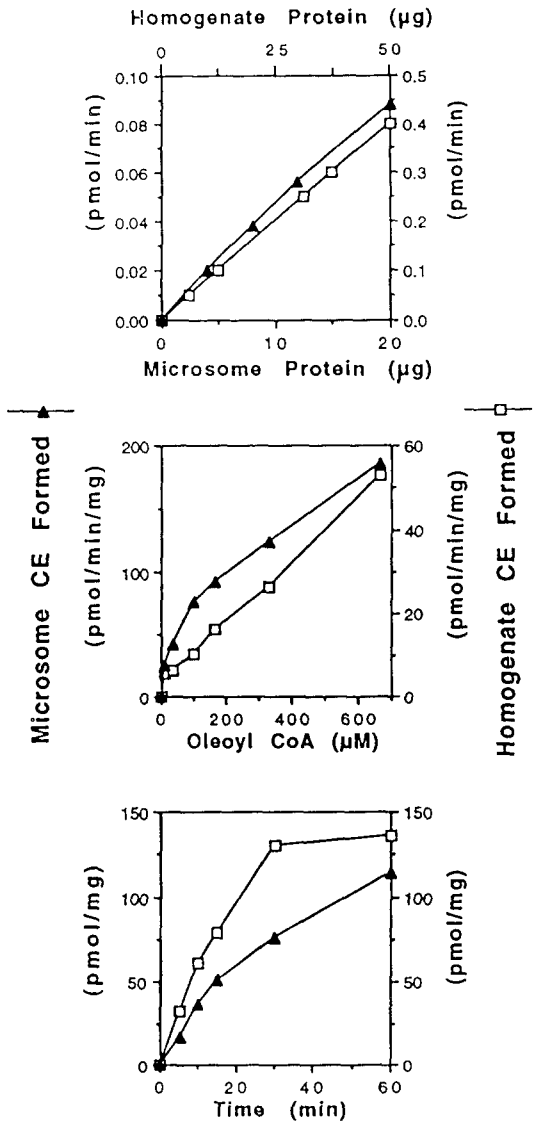

Fig. 1. Characteristics of the assay for ACAT activity using either hamster liver homogenates or microsomes as enzyme preparations. Liver homogenates and microsomes were prepared from hamsters that had been maintained on a 0.5% cholesterol diet for 7 days. All incubations were performed at 37° for 15 min using 10 μM oleoyl CoA and 50 μg homogenate protein (□) or 12.5 μg microsome protein (▲) unless otherwise indicated. (Top) effect of homogenate or microsome protein. (Middle) effect of oleoyl CoA concentration. (Bottom) effect of incubation time. For each assay, a 15 μL aliquot of each incubation was spotted directly onto a silica gel G thin-layer plate, and the lipids were separated as described in Materials and Methods. Each point represents the average of duplicate determinations.

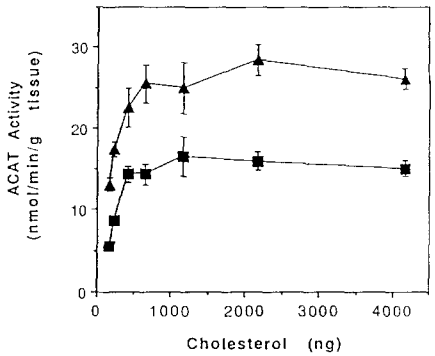

Fig. 2. Effect of exogenously added cholesterol on ACAT activity from control and LE-treated hamsters. Hamsters were cholesterol fed for 7 days, and the livers were obtained, homogenized and assessed for ACAT activity. The control animals (▲) did not receive drug treatment, whereas LE-treated animals (■) received 50 mg LE/kg/day by oral gavage. The data obtained at the lowest cholesterol concentration represent the activity when only the microsome cholesterol and no additional cholesterol was present. Cholesterol concentrations in the microsomes were assessed by HPLC analysis. Increasing cholesterol levels in the incubations were obtained by adding the appropriate amount to adjust the total incubation cholesterol content from 1-μl aliquots of concentrated stock solutions in acetone. Data are shown as means ± SEM (N = 6).

To establish the potential effects of LE on hepatic ACAT activity, experiments were performed in which additional cholesterol was added to the homogenates. The addition of exogenous cholesterol was assessed in order to overcome any effects LE may have on hepatic free cholesterol availability based upon effects on cholesterol absorption in the intestine. Figure 2 shows the ACAT activity measured in homogenates from cholesterol-fed animals as a function of the amount of free cholesterol in each homogenate. The first data point for each curve represents the amount of free cholesterol in the homogenate from the animal as measured by HPLC. Increasing amounts of cholesterol were then added from a small volume of acetone as described in Materials and Methods. For the cholesterol-fed, control animals, exogenously added free cholesterol caused an increase in ACAT with a saturation of approximately twice that observed in native homogenates. Homogenates from animals treated with LE displayed a similar profile although the absolute amount of cholesteryl ester formed was decreased at all concentrations of cholesterol in the incubations. The addition of cholesterol was unable to restore ACAT activity to levels observed in non-treated animals. Taken together, these data suggest that LE has the ability to modulate ACAT activity by a mechanism that does not involve changes in the cholesterol substrate pool.

The ability of LE to inhibit homogenate ACAT activity is shown in Fig. 3. These assays were performed using homogenates from both chow and cholesterol-fed animals since it is likely that increased lipid associated with the homogenates from cholesterol-fed animals may alter the activity of LE. In addition, assays were performed in the presence or absence of a saturating level of exogenously added cholesterol. For both the chow- and cholesterol-fed

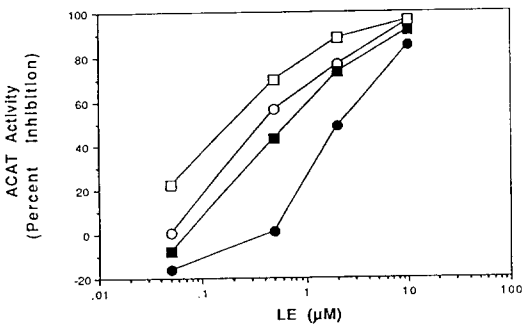

Fig. 3. Effect of LE concentration on liver homogenate ACAT activity. Liver homogenates were prepared from chow- and cholesterol-fed hamsters after 1 week of dietary treatment. LE was then added at the indicated concentrations, and ACAT activity was assessed in the absence and presence of an additional 1 μg exogenous cholesterol. Key: ACAT activity in homogenates from chow-fed hamsters in the absence of added cholesterol (●), and in the presence of exogenous cholesterol (○); ACAT activity in homogenates from cholesterol-fed hamsters in the absence of added cholesterol (■), and in the presence of exogenous cholesterol (□). ACAT activity in the absence of LE was 12.7 and 34.8 nmol/min/g tissue for the homogenates from chow-fed animals in the absence and presence of exogenous cholesterol, respectively. ACAT activity in the absence of LE was 13.4 and 32.8 nmol/min/g tissue for the homogenates from cholesterol-fed animals in the absence and presence of exogenous cholesterol, respectively. Each data point represents the average of duplicate determinations.

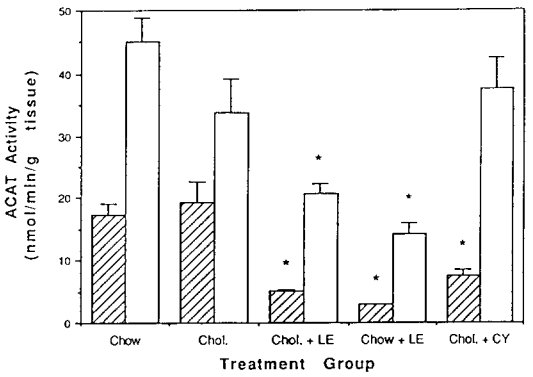

Fig. 4. Effect of drug treatment on liver homogenate ACAT activity in the absence and presence of exogenous cholesterol. Animals were fed either the chow diet or the cholesterol-containing diet for 7 days. As indicated, several of the groups also received 50 mg/kg/day of LE by oral gavage and one group received cholestyramine (CY, 2.5% of diet) mixed in the cholesterol-containing diet. Liver homogenates were prepared, and ACAT activity was measured both in the absence (control, stippled bars) and in the presence of 1 μg of exogenously added cholesterol (open bars). Each group contained six animals. The error bars represent the SEM, and significance ($P < 0.05$) relative to the diet control is indicated by an asterisk (*).

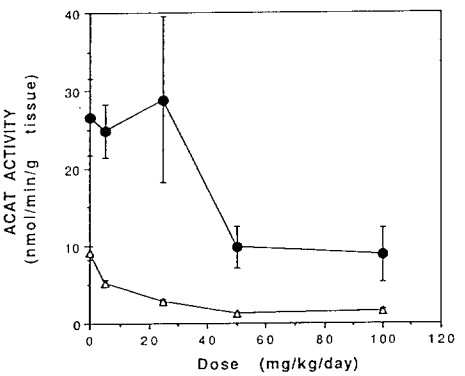

Fig. 5. Effect of oral LE dose on liver homogenate ACAT activity from hamsters in the absence and presence of exogenous cholesterol. Cholesterol-fed hamsters were treated with the indicated levels of LE for 7 days. Liver homogenates were then prepared and assayed for ACAT activity under control conditions without additional cholesterol (△) or in the presence of an additional 1 μg cholesterol (●). Data are shown as means ± SEM (N = 4/group).

animals, LE caused potent inhibition of ACAT activity, reaching nearly 100% inhibition at concentrations near 10 μM. Small variations in the potency of LE were observed in the different preparations, although the data suggest that LE potency may be increased by the addition of exogenous cholesterol. In a similar assay, performed using liver microsomes from chow-fed hamsters, an IC_{50} value of approximately 2 μM was observed. Taken together, these data demonstrate that LE is an effective inhibitor of ACAT activity under a variety of experimental conditions and that LE has the potential to eliminate nearly all of the cholesterol esterification in the liver.

The ability of LE to affect ACAT activity in homogenate preparations from both chow- and cholesterol-fed animals is shown in Fig. 4. Treatment of hamsters maintained on either diet with LE resulted in decreased ACAT activity in both the absence and presence of exogenously added cholesterol as compared with the appropriate diet control. In the absence of exogenously added cholesterol, the inhibition of cholesterol absorption with the non-absorbed bile acid sequestrant cholestyramine caused a decrease in ACAT activity. In contrast to animals treated with LE, ACAT activity in homogenates from cholestyramine-treated hamsters could be restored to the level observed in cholesterol-fed control animals with exogenous cholesterol addition. The recovery of activity by exogenous cholesterol in the cholestyramine-treated animals suggests that this agent reduces ACAT activity by limiting the free cholesterol availability in the liver for esterification. Taking these data together suggests that ACAT activity in LE-treated animals was decreased due to direct inhibition of the enzyme by the ACAT inhibitor.

The level of hepatic ACAT activity in hamsters treated with different amounts of LE is shown in Fig. 5. In the absence of added cholesterol, ACAT

activity decreased in a dose-proportional manner. A similar decreasing trend in ACAT activity was also observed in the incubations containing exogenous cholesterol, resulting in a 69% decrease in activity when hamsters received 100 mg/kg of LE.

DISCUSSION

The role of hepatic ACAT in cholesterol metabolism differs between species. In some animals such as the hamster, dietary cholesterol feeding causes large amounts of cholesteryl ester to be formed and stored within hepatocytes as intracellular lipid droplets [23]. In other animals such as the rhesus monkey, hepatic cholesteryl esters do not accumulate but are secreted into plasma as lipoproteins [24]. In addition to the effects of ACAT inhibitors on cholesterol absorption, effects at the level of the liver may be important for the overall therapeutic action of this class of compounds. In this report, a kinetic paradigm has been established that demonstrates the direct inhibitory activity of a well studied ACAT inhibitor at the level of the liver enzyme. The data demonstrate that ACAT activity can be suppressed upon oral administration of an ACAT inhibitor in a manner that is inconsistent with a decreased cholesterol substrate in the liver due to decreased cholesterol absorption.

Assessments of ACAT kinetic parameters have been hampered due to the lack of a pure protein and the insoluble nature of the substrates. For these reasons, kinetic constants from Michaelis–Menten analysis have been avoided. Several groups have reported that acyl CoA can cause inhibition of ACAT activity at higher concentrations; however, the inhibition observed can be attenuated by including albumin [25–27], which has a high affinity binding site for acyl CoA [28]. To observe effects on cholesterol availability and minimize detergent properties of acyl CoA, we chose to use 10 μM oleoyl CoA, a moderately low level without indications of substrate-induced inhibition. Addition of exogenous cholesterol to membrane fractions has been used by several groups in the past to estimate maximal rates of cholesterol esterification at constant levels of acyl CoA and albumin. Some have used detergents to add cholesterol [9, 10]; however, our laboratory has found significant inhibition of ACAT activity at very low levels of detergent (data not shown). We therefore used small volumes of organic solvent to add exogenous cholesterol as has been done previously [25, 29, 30]. Taken together, these results indicate that ACAT esterifying potential can be assessed despite the use of insoluble substrates at submaximal concentrations.

The direct effect of the ACAT inhibitor CL277082 (LE) on the liver indicates that the compound is absorbed in the hamster and is likely transported to the liver. Pharmacological studies suggest that the major effect of LE is likely to be due to the inhibition of cholesterol absorption, since in the rat LE treatment causes a 3-fold increase in hepatic HMG CoA reductase activity [31], indicating that the reduced cholesterol delivery to the liver has derepressed cholesterol biosynthesis. This report demonstrates a direct hepatic effect of ACAT inhibition; however, the paradigm does not allow discrimination of the intestinal effects of LE from hepatic effects as related to the hypocholesterolemic action of the compound.

The ability of cholestyramine-treated animals, in which the liver had been deprived of dietary cholesterol, to restore full ACAT activity upon the addition of exogenous cholesterol strongly suggests that cholesterol deprivation in the liver does not down-regulate ACAT activity. These data are in agreement with the results of others as reviewed by Suckling and Stange [1], suggesting that ACAT activity is responsive to the amount of free cholesterol present in the liver. ACAT activity assessed in tissue homogenates was only observed at about half-maximal levels, demonstrating the potential of the liver to esterify an influx of free cholesterol. These observations are further supported by the mass of free and esterified cholesterol found in the livers. Free cholesterol levels remain essentially constant despite dietary or drug treatment, while very large accumulations of cholesteryl ester were observed.

The data from this study suggest that the hypocholesterolemic effect of ACAT inhibitors can be due to a combination of at least two possible mechanisms. In addition, this study provides a paradigm by which other ACAT inhibitors may be assessed for potential inhibition of hepatic ACAT. Inhibition of dietary cholesterol absorption reduces cholesterol delivery to the liver in dietary lipoproteins, thereby reducing cholesterol esterification due to reduced cholesterol substrate for the ACAT reaction. In addition, the data demonstrate that direct inhibition of hepatic ACAT may also be a factor causing decreased synthesis of cholesteryl esters. A correlation between ACAT activity in the liver and plasma LDL cholesteryl esters has been demonstrated in perfused non-human primate livers [3, 32]. Further evidence for ACAT activity regulating cholesteryl ester content of lipoproteins has been obtained by analyzing lipoproteins secreted by cultured hepatocytes [33, 34]. In studies employing perfused rat livers, depletion of hepatic cholesterol by lovastatin treatment reduced VLDL lipid secretion, suggesting a role for free cholesterol in regulating hepatic lipoprotein secretion. In the hamster, we have reported previously a decrease in VLDL cholesterol in ACAT-inhibited animals and noted that the cholesteryl esters become more polyunsaturated, suggesting that a greater proportion of these esters are derived from the LCAT reaction [23]. In a recent report, Krause and coworkers [4] employed a variety of pharmacological interventions to suggest hepatic ACAT activity is reduced upon administration of another ACAT inhibitor, CI-976. Based upon the cholesterol and cholesteryl ester content of the liver upon ACAT inhibition, they speculated that excess sterol is secreted into bile either as cholesterol or bile acids, depending upon the duration of drug treatment. The data from this report support this hypothesis since free cholesterol levels were not increased, even under conditions where hepatic ACAT was directly inhibited.

In summary, the effects of oral ACAT inhibitors on plasma lipid levels were consistent with an effect

of the compound on intestinal cholesterol absorption as well as a direct hepatic effect of the compound.

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