ACAT Inhibition Decreases LDL Cholesterol in Rabbits Fed a Cholesterol-Free Diet
Marked Changes in LDL Cholesterol Without Changes in LDL Receptor mRNA Abundance

Brian R. Krause, Michael E. Pape, Karen Kieft, Bruce Auerbach, Charles L. Bisgaier, Reynolds Homan, Roger S. Newton

Abstract

Rabbits fed low-fat, cholesterol-free diets containing casein as the sole protein source develop endogenous hypercholesterolemia (EH). To test the hypothesis that lipoprotein cholesteryl esters in EH rabbits are acyl coenzyme A:cholesterol acyltransferase (ACAT) derived, we treated EH rabbits with CI-976, a potent and selective ACAT inhibitor. In addition, since cholesterol and bile acid synthesis as well as low-density lipoprotein (LDL) receptor activity are reduced in EH rabbits, we determined whether changes in gene expression for 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, 7α-hydroxylase, and the LDL receptor might be associated with the efficacy due to ACAT inhibition. Compared with EH controls, CI-976–treated rabbits (50 mg/kg per day for 5 weeks) had decreased plasma total cholesterol (−43%), very-low-density lipoprotein (VLDL) cholesterol (−62%), LDL cholesterol (−43%), plasma apolipoprotein B (−23%), liver cholesteryl esters (−39%), LDL size, VLDL and LDL cholesteryl ester content (percent of total lipids), cholesteryl oleate/cholesteryl linoleate ratios in VLDL and LDL (25% to 30%), and ex vivo liver ACAT activity. The triglyceride/cholesteryl ester ratio increased twofold to fourfold in these apolipoprotein B–containing lipoproteins. Endogenous cholesterol absorption appeared to be unaffected by drug treatment. CI-976 failed to alter specific hepatic mRNAs involved in cholesterol metabolism, but comparisons among dietary control groups revealed a marked reduction in 7α-hydroxylase mRNA, no change in LDL receptor mRNA, and an increase in HMG-CoA reductase mRNA in EH rabbits compared with normal chow-fed controls. In cholesterol-fed rabbits (exogenous hypercholesterolemia) mRNA levels for all three proteins were lower than in chow-fed rabbits, especially that for HMG-CoA reductase. We conclude that (1) EH is due to the combined effects of hepatic hypersecretion of ACAT-derived lipoproteins and the relative absence of 7α-hydroxylase gene expression; (2) ACAT inhibition in this animal model results in a population of LDL particles that are fewer in number and smaller than those found in EH controls; and (3) marked changes in plasma LDL cholesterol can occur by diet (EH) or drug treatment (ACAT inhibitor) in rabbits in the absence of changes in LDL receptor gene expression. (Arterioscler Thromb. 1994;14:598-604.)

Key Words • cholesterol • acyl coenzyme A:cholesterol acyltransferase • LDL cholesterol • cholesteryl esters • apolipoprotein B • LDL receptor mRNA • 7α-hydroxylase mRNA • 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA

An endogenous hypercholesterolemia (EH) can be produced experimentally in rabbits by feeding a cholesterol-free, purified diet containing casein as the sole protein source.1-3 Unlike cholesterol-fed rabbits or Watanabe heritable hyperlipidemic rabbits, there is a selective increase in the cholesteryl concentration of low-density lipoprotein (LDL) in the EH rabbit and no change in plasma triglycerides. Therefore, the plasma lipid and lipoprotein profile of the EH rabbit phenotypically resembles human type IIa hyperlipoproteinemia.4

Although a reduction in receptor-mediated catabolism of LDL is thought to be responsible in part for the EH condition in rabbits,5-7 increased LDL production may also contribute. Perfused livers from EH rabbits secrete an increased number of cholesteryl ester–rich very-low-density lipoprotein (VLDL) particles.8 Evidence for the direct secretion of intermediate-density lipoprotein and LDL that contains more cholesteryl ester has also been presented.9 Since the cholesteryl oleate/linoleate ratio in these apolipoprotein (apo) B–containing particles is elevated, these cholesteryl esters may be derived from hepatic acyl coenzyme A:cholesterol acyltransferase (ACAT).8 The present experiments were designed to test this possibility by comparing control EH rabbits with EH rabbits treated with the potent, selective ACAT inhibitor CI-976.9-11 In addition, since LDL receptor activity, bile acid synthesis, and cholesterol synthesis are lower in EH rabbits than in cholesterol-fed rabbits (exogenous hypercholesterolemia) mRNA levels for all three proteins were lower than in chow-fed rabbits, especially that for HMG-CoA reductase. We conclude that (1) EH is due to the combined effects of hepatic hypersecretion of ACAT-derived lipoproteins and the relative absence of 7α-hydroxylase gene expression; (2) ACAT inhibition in this animal model results in a population of LDL particles that are fewer in number and smaller than those found in EH controls; and (3) marked changes in plasma LDL cholesterol can occur by diet (EH) or drug treatment (ACAT inhibitor) in rabbits in the absence of changes in LDL receptor gene expression. (Arterioscler Thromb. 1994;14:598-604.)

Key Words • cholesterol • acyl coenzyme A:cholesterol acyltransferase • LDL cholesterol • cholesteryl esters • apolipoprotein B • LDL receptor mRNA • 7α-hydroxylase mRNA • 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA

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therefore, in the present study, we used a fecal isotope method to
determine the lipid composition of lipoproteins by using a
semi-automated microtiter plate system and commercial re-
determine in isopropanol extracts as described. Whole-
rat muscle RNA was isolated from the CI-976 diet was reduced in the drug-treated
rat liver RNA as described in detail elsewhere, uses standard RNA that
altered the results. Moreover, if such losses did

Results

Body weight gain did not differ between treatment
groups in the first experiment (19.5±5.6% for controls and
27.8±4.7% for the CI-976 group) (Tables 2 and 3 and Figs 1 and 2) or in subsequently repeated experiments in which
mRNAs (Table 4), cholesterol absorption (Fig 3), or other parameters were determined. Liver wet weights were
62.76±3.19 g for controls and 59.46±4.25 g for the CI-976
group (−5%; not significant). CI-976 significantly lowered plasma cholesterol (−43%) and apoB (−23%) concen-
trations without changing plasma triglycerides (Table 2).
Liver total cholesterol was reduced in the drug-treated
group (−18%) because of changes in both cholesteryl
esters (−39%) and free cholesterol (−13%), but the latter
was only marginally significant (P<.1; Table 2).

The average high-performance gel chromatography profiles of plasma lipoproteins for the two treatment
groups are depicted in Fig 1, and the mean absolute values for both groups are shown in Fig 2. In control EH
rabbits the majority of the cholesterol (86%) was trans-
port ed in particles that were comparable in size to
human LDL. Very few VLDL-sized (8%) or HDL-

It has been stated that cholesterol absorption cannot be
determined in rabbits by the dual-isotope technique; therefore, in the present study, we used a fecal isotope technique to
assess the efficiency of absorption of endogenous cholesterol in EH rabbits. Briefly, [3H]cholesterol was administered orally by
gavage immediately before the meal, and [3H] nonsaponifiable lipids (NSL) in feces were measured over an 8-day period. The
oral dose was prepared by adding the isotope (730 μCi, 16
μmol, in 30 μL isopropanol) to rat bile (0.75 mL), followed by
brief bath sonication and dilution into 19 vols 0.9% NaCl
containing 5% glucose. Each animal received exactly the same
amount of radiolabeled cholesterol. [14C]β-sitosterol was not
used as an internal standard to correct for degradation losses of
[3H]cholesterol because this correction in rabbits does not
significantly alter the results. Moreover, if such losses did
occur there is no reason to suspect that they would be different
in the two treatment groups, i.e., the assumption is made that
CI-976 itself does not contribute to degradation loss of neutral sterols.

RNA Analysis

At the time of death liver tissue was removed and immedi-
ately frozen in liquid nitrogen. Total RNA was isolated from
these tissues as described and the concentration was
determined by absorbance at 260 nm; the 260 nm/280 nm ratio
of all samples was 1.9 to 2.0. To measure mRNA levels for
LDL receptor, 7a-hydroxylase, and HMG-CoA reductase,
rabbit-specific cDNAs for each gene were isolated by using the
polymerase chain reaction. These cDNAs served as tem-
plates to synthesize antisense RNA probes that were then used
in an RNase protection assay. This solution-based assay,
described in detail elsewhere, uses standard RNA that
allows relatively precise quantification of specific gene tran-
scripts. To quantify specific mRNA levels for the genes of
interest, 50 μg total liver RNA and 10 to 20 pg internal
standard were used in each hybridization reaction.

Drug-treated Groups. They were meal-fed the diets described
in Table 1 (50 to 70 g/d, 8 am to 12 pm daily for 5 to 6 weeks;
Research Diets, Inc). Under these conditions the animals consuming
the CI-976 diet received a dose of approximately 50 mg/kg per day. In a separate experiment, groups of rabbits
were also fed normal chow or chow supplemented with 0.5%
Wheat starch and to

**Table 1.** Composition of Endogenous Hypercholesterolemic Rabbit Diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Control, g/kg</th>
<th>CI-976, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>270</td>
<td>270</td>
</tr>
<tr>
<td>α-Methionine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Wheat starch</td>
<td>343</td>
<td>341.6</td>
</tr>
<tr>
<td>Molasses</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Cellulose</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Corn oil</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Salt mix*</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Vitamin mix*</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>CI-976</td>
<td>0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Total 1000 1000

* AIN-76A mixes.

**Table 2.** Effect of CI-976 on Plasma Lipids, ApoB, and Liver Cholesterol in EH Rabbits

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Plasma Cholesterol, mg/dL</th>
<th>Plasma Triglycerides, mg/dL</th>
<th>Plasma ApoB, AU</th>
<th>Liver Cholesterol, mg/Organ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>297±37</td>
<td>69±12</td>
<td>100.0±11.3</td>
<td>170±9</td>
</tr>
<tr>
<td>CI-976</td>
<td>170±18†</td>
<td>69±7</td>
<td>76.8±7.2*</td>
<td>140±7†</td>
</tr>
</tbody>
</table>

Apo B indicates apolipoprotein B; EH, endogenous hypercholesterolemic. Values are expressed as mean±SEM; n=12 rabbits per group.

†P<.05; †P<.01; †P<.005.
TABLE 3. Lipid Composition of ApoB-Containing Lipoproteins in EH Rabbits

<table>
<thead>
<tr>
<th></th>
<th>VLDL</th>
<th>LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EH</td>
<td>EH+CI-976</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>26.6±3.5</td>
<td>55.4±4.3*</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>18.6±0.5</td>
<td>17.6±0.8</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>11.5±0.7</td>
<td>8.5±0.3†</td>
</tr>
<tr>
<td>Cholesteryl esters</td>
<td>43.3±2.5</td>
<td>18.4±3.9*</td>
</tr>
<tr>
<td>18:2</td>
<td>31.4±2.3</td>
<td>37.8±1.3*</td>
</tr>
<tr>
<td>18:1</td>
<td>68.6±2.3</td>
<td>62.2±1.3*</td>
</tr>
<tr>
<td>Ratio (18:1/18:2)</td>
<td>2.18</td>
<td>1.65</td>
</tr>
<tr>
<td>Triglycerides/cholesteryl esters</td>
<td>0.66±0.13</td>
<td>2.66±0.49†</td>
</tr>
<tr>
<td>Cholesteryl esters+triglycerides, % volume</td>
<td>69.9±1.0</td>
<td>73.4±0.8*</td>
</tr>
</tbody>
</table>

ApoB indicates apolipoprotein B; EH, endogenous hypercholesterolemic; VLDL, very-low-density lipoprotein; and LDL, low-density lipoprotein. Values are percent of total lipids for eight animals/group (mean±SEM). For cholesteryl linoleate (18:2) and cholesteryl oleate (18:1) the values for each are divided by the sum of these two esters (ie, other cholesteryl esters were not measured).

*P<.05; †P<.001.

Changes in the lipid composition of HDL were observed (data not shown). The percent mass of cholesteryl esters decreased at the expense of triglycerides in both VLDL and LDL in the CI-976 group. In EH control rabbits cholesteryl esters represented 43% and 52% of the lipid mass in VLDL and LDL, respectively. CI-976 reduced the percentages of cholesteryl esters in VLDL and LDL to 18% and 45%, respectively, while the amount of triglyceride increased in both lipoproteins. These changes in total cholesteryl esters were associated with changes in the fatty acid composition of the cholesteryl esters, as shown by significant decreases in the cholesteryl oleate/cholesteryl linoleate ratios (25% to 31%) in the drug-treated animals. The shift in core lipids was also illustrated by the fact that the calculated triglyceride/cholesteryl ester ratio increased fourfold in VLDL and twofold in LDL with drug treatment. These changes are in the direction of values reported by many laboratories for chow-fed rabbits (VLDL triglyceride/cholesteryl ester range, ~4 to 28; LDL triglyceride/cholesteryl ester range, 0.5 to 1.4). The surface (cholesterol, phospholipid)—to—core (cholesteryl esters, tri-
glyceride) lipid mass ratio was significantly higher in the LDL from CI-976-treated rabbits (0.741 ± 0.021 in controls versus 0.801 ± 0.012 in the CI-976 group, P = .0275), again suggesting that the LDL in the drug-treated group was, on average, smaller than in control rabbits. This estimate assumes an equal amount of protein (apoB) per LDL particle. Due in part to a significant decrease in the amount of VLDL free cholesterol, the surface/core lipid mass ratios for VLDL were also different between treatment groups, but in the opposite direction (0.430 ± 0.021 in controls versus 0.367 ± 0.016 in CI-976-treated rabbits, P = .0325). Since the assumption cannot be made that the percentage of protein was similar in the VLDL particles from these groups, statements regarding the size of VLDL cannot be made on this basis. However, if the sizes of the lipoproteins are estimated from the volume percentage of the major nonpolar lipid components as described by Havel et al,27 then the VLDL does appear to be larger and the LDL smaller in drug-treated EH rabbits (Table 3). These size estimates for LDL were confirmed in a separate experiment by using nondenaturing gradient gel electrophoresis (2% to 16% gels). The average sizes of LDL were smaller in drug-treated EH rabbits (Table 3). These size estimates for LDL were confirmed in a separate experiment by using nondenaturing gradient gel electrophoresis (2% to 16% gels). The average sizes of LDL were

Cholesterol absorption was estimated by administering [3H]cholesterol orally and analyzing the fecal excretion of 3H radioactivity in NSL as a function of time. Plasma cholesterol concentrations averaged 273 mg/dL in controls and 119 mg/dL in the CI-976 group (−56%) in this repeated experiment. There was significantly more [3H]NSL in the feces of drug-treated animals at days 5 and 6 but not at other time points (Fig 3). Total areas under the curves were not significantly different between groups (22.06 ± 1.73 for controls versus 25.52 ± 2.25 for CI-976-treated rabbits, P = .287).

The mRNA levels for LDL receptor, 7α-hydroxylase, and HMG-CoA reductase were determined in EH rabbits with and without CI-976 treatment, and these values were compared for reference with those of chow-fed rabbits and cholesterol-fed rabbits (exogenous hypercholesterolemia) (Table 4). In this repeated experiment the plasma cholesterol concentrations were 443, 226, 64, and 613 mg/dL for the EH control, CI-976-treated EH, chow-fed, and cholesterol-fed groups, respectively. The percentages of cholesterol transported in LDL were 86%, 85%, 35%, and 56%, respectively. CI-976 had no significant effects on the levels of these specific hepatic mRNAs in EH rabbits. However, significant diet-related differences were noted among the other treatment groups. Compared with normal chow-fed controls, in EH rabbits levels of LDL receptor mRNA were unchanged, 7α-hydroxylase mRNA was drastically reduced, and mRNA levels for HMG-CoA reductase were increased threefold. Cholesterol feeding, on the other hand, decreased LDL receptor mRNA (−44%), HMG-CoA reductase mRNA (more than −90%), and 7α-hydroxylase mRNA (−58%) compared with chow-fed controls. The latter was significant only at the P < .1 level owing to the inclusion of one chow-fed rabbit with undetectable mRNA for 7α-hydroxylase. If this animal is omitted from the chow group, the decrease in 7α-hydroxylase mRNA (−65%) becomes more significant (4.14 ± 0.89 pg/μg total mRNA for chow-fed versus 1.45 ± 0.62 pg/μg total mRNA for cholesterol-fed, P = .0381). This outlier had a typical plasma cholesterol level for a chow-fed rabbit (43 mg/dL), and its mRNAs for the other proteins were close to the mean. It is clear from these data that EH rabbits had a markedly different mRNA profile than exogenous hypercholesterolemic rabbits.

Table 4. LDL Receptor, 7α-Hydroxylase, and HMG-CoA Reductase mRNA Levels in EH Rabbits

<table>
<thead>
<tr>
<th>Treatment Group (n)</th>
<th>LDL Receptor mRNA</th>
<th>7α-Hydroxylase mRNA</th>
<th>HMG-CoA Reductase mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>EH (6)</td>
<td>0.55 ± 0.08†</td>
<td>&lt;0.1*</td>
<td>1.21 ± 0.32†</td>
</tr>
<tr>
<td>EH + CI-976 (5)</td>
<td>0.64 ± 0.43</td>
<td>&lt;0.1*</td>
<td>1.22 ± 0.63</td>
</tr>
<tr>
<td>Chow fed (6)</td>
<td>0.63 ± 0.17</td>
<td>3.45 ± 1.00</td>
<td>0.41 ± 0.07</td>
</tr>
<tr>
<td>Cholesterol fed (5)</td>
<td>0.35 ± 0.04†</td>
<td>1.45 ± 0.62</td>
<td>&lt;0.1*</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; and EH, endogenous hypercholesterolemic. Values for mRNAs are expressed as picograms per microgram total mRNA (mean ± SEM). Cholesterol-fed rabbits were fed the diet described in Reference 12.

* Only one animal had detectable levels.
† P < .05 vs chow-fed controls.
‡ P < .05 vs cholesterol-fed controls.
§ P < .10 vs chow-fed controls.

24.2 ± 0.2 and 23.7 ± 0.15 nm in EH control rabbits and CI-976-treated EH rabbits, respectively (P = .05, n = 3 rabbits per group).

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Discussion

Etiology of the EH Condition

A major conclusion from the present study relates to the etiology of the hypercholesterolemia in EH rabbits. Cholesterol and bile acid synthesis as well as fecal neutral steroid and bile acid excretion are reduced in EH rabbits compared with Chow-fed controls. In addition, Cha et al. reported that LDL receptor activity is markedly decreased in liver membranes from EH rabbits compared with those from Chow-fed rabbits and that this result was decreased in vivo catabolism of LDL. Khosla et al. have definitively shown that casein is the dietary component responsible for the decreased fractional catabolic rate of LDL in EH rabbits by comparing casein-fed with soy-fed animals. However, the production rate of LDL apoB is also increased 2.5-fold. This latter observation is consistent with earlier work showing that perfused livers from EH rabbits secrete more apoB-containing particles. The fact that the perfusate lipoproteins were enriched in cholesteryl oleate, the major ACAT product, suggests that there was no preferential uptake of cholesteryl oleate over cholesteryl palmitate.

The present results with the specific ACAT inhibitor CI-976 provide further evidence that the livers from EH rabbits secrete increased amounts of ACAT-derived cholesteryl esters. CI-976 treatment lowered plasma cholesterol and liver cholesteryl ester concentrations. Moreover, the cholesteryl oleate/cholesteryl linoleate ratios in both VLDL and LDL were increased by drug treatment, and ex vivo liver ACAT activity was inhibited (see below). Taken together, available data now suggest that the increase in the absorption of endogenous cholesterol and bile acids that occurs in EH rabbits results in an increase in liver cholesterol that leads to inhibition of de novo cholesterol synthesis, downregulation of LDL receptor activity, and an increase in ACAT substrate. The increase in bile acid absorption causes feedback inhibition of bile acid synthesis in the liver and contributes to the elevation of hepatic cholesterol. The majority of the accumulated free cholesterol is esterified by ACAT, since dietary proteins do not influence the biliary secretion of cholesterol or bile acids. Although difficult to quantify precisely, these liver cholesteryl esters appear to be a major source of the cholesteryl esters in plasma apoB-containing lipoproteins (VLDL, intermediate-density lipoprotein, and LDL; see Reference 8) in EH rabbits. The critical role of ACAT in this sequence of events is further illustrated with CI-976 as a pharmacological tool and can be readily incorporated into comprehensive descriptions of this animal model.

Intestinal Versus Liver ACAT in EH Rabbits

Some experimental drugs (eg, surformer) lower LDL cholesterol substantially in animals fed cholesterol-free diets, and CI-976 inhibits endogenous cholesterol absorption in the rat lymph fistula model. The effect of CI-976 on cholesterol absorption in EH rabbits was therefore determined, since inhibition of intestinal ACAT could contribute to the efficacy observed in this study. CI-976 had no major effect on endogenous cholesterol absorption, as shown by higher excretion of orally administered radiolabeled cholesterol on only two separate days after dosing radiolabeled cholesterol and no change in the calculated areas under the curve. This small drug effect was noted after the bulk of the label had been excreted and probably represents [3H]-cholesterol that had undergone at least one enterohepatic circulation. On the basis of these and other data (eg, 18:1/18:2 ratios in LDL cholesteryl ester and liver cholesteryl ester content), we favor the view that liver ACAT is the major drug target for CI-976 that is responsible for the efficacy in EH rabbits and that inhibition of the intestinal enzyme in these animals on a cholesterol-free diet does not contribute significantly to plasma cholesterol-lowering activity. This was recently confirmed in a separate experiment. ACAT activity measured exactly as described was inhibited by 73% in livers from nonfasted EH rabbits treated with CI-976 (Anderson and Homan, unpublished data, 1993).

Efficacy of ACAT Inhibitors Compared With Other Agents

The current results illustrate that efficacy in terms of plasma cholesterol reduction due to ACAT inhibition is not limited to cholesterol-fed animal models. In other words, certain ACAT inhibitors, depending on the degree of hepatic drug exposure, drug metabolism, and/or drug selectivity, may lower plasma cholesterol and apoB by mechanisms other than inhibition of exogenous cholesterol absorption or hepatic esterification of diet-derived cholesterol. This has also been exemplified in hamsters, in which CI-976 significantly lowered VLDL cholesterol in animals fed a cholesterol-free, purified diet. Interestingly, clofibrate and other fibrates do not lower plasma cholesterol in EH rabbits, whereas drugs that result in enhanced hepatic LDL receptor activity (eg, resins, HMG-CoA reductase inhibitors) are active.

Specificity of CI-976

It is worth mentioning that CI-976 has thus far proven to be specific for ACAT with regard to lipid-regulating mechanisms. For example, it has no effect on the activities of HMG-CoA reductase, fatty acyl-CoA hydrolase, triglyceride synthetase, pancreatic cholesteryl ester hydrolase, lecithin:cholesterol acyltransferase, or acyl-CoA:monoglyceride acyltransferase. The CI-976 also has no effect on LDL receptor activity in vitro (Bocan, Ferguson, Sekerke, and Krause, unpublished data, 1990). As yet we have not determined whether CI-976 alters 7α-hydroxylase activity in vitro or in vivo, but a transient increase in biliary bile acid secretion is observed in cholesterol-fed rats treated with CI-976. Therefore, we cannot rule out stimulation of this enzyme activity, directly or indirectly, as a possible mechanism in the present study, although it seems unlikely in view of the mRNA data (see below).

Association Between ACAT, ApoB, and LDL Size

With respect to efficacy in EH rabbits, it should also be noted that plasma apoB, presumably mostly apoB-100, was decreased by CI-976, as reported in cholesterol-fed and sucrose-fed rats. Experiments in HepG2 cells have implicated ACAT as a possible regulator of apoB secretion, and others have provided evidence that a reduction of cholesteryl esters may accelerate the intracellular degradation of apoB. In addition, Carr and Rudel have shown that CI-976 decreases cho-
Hepatic mRNAs: CI-976-Treated EH Rabbits Versus Chow- and Cholesterol-Fed Rabbits

Since liver cholesterol concentration is elevated in EH rabbits compared with chow-fed or soy protein-fed rabbits,\(^3\),\(^4\) and we have previously observed decreases in liver cholesterol with CI-976 in other animal models,\(^5\),\(^6\),\(^7\) we examined possible drug-related changes in the expression of genes involved in lipid/ lipoprotein metabolism, the activities of which are known to be sensitive to changes in cholesterol balance across the liver (ie, LDL receptor, \(\alpha\)-hydroxylase, and HMG-CoA reductase). Moreover, the activities of these proteins are decreased in EH rabbits,\(^8\),\(^9\) which prompted us to determine possible changes due to CI-976 at the molecular level.

LDL lowering due to CI-976 in EH rabbits occurred in the absence of any changes in these specific mRNAs. But since chow-fed and cholesterol-fed (exogenous hypercholesterolemia) groups were included for comparison, this experiment did reveal novel changes related to the type of diet. To our knowledge, there are no data available on the expression of these genes in EH rabbits. Three major observations are worth noting. Compared with chow-fed controls, LDL receptor mRNA levels were unchanged, \(\alpha\)-hydroxylase mRNA levels were drastically reduced, and mRNA abundance for HMG-CoA reductase was increased. From these data it appears that the mechanism for decreased LDL receptor activity in EH rabbits\(^10\),\(^11\) is not decreased gene transcription (assuming that degradation of the mRNA is unchanged). Moreover, the EH condition may be due in part to very low rates of \(\alpha\)-hydroxylase gene expression. This may be secondary to the increased flux of bile acids to the liver in EH rabbits,\(^12\),\(^13\) since dietary cholate decreases \(\alpha\)-hydroxylase mRNA in other species.\(^14\) Finally, it is likely that HMG-CoA reductase activity is reduced in EH rabbits\(^4\),\(^15\) despite increased abundance of mRNA (Table 4), providing further evidence for the multiple levels of regulation of this important rate-limiting enzyme. These changes in mRNAs in EH rabbits are distinctly different from those observed in cholesterol-fed rabbits (decrease in mRNAs for all three proteins), which in turn are different from those reported for cholesterol-fed rats; eg, there is little\(^16\) or no\(^17\) reduction in HMG-CoA reductase mRNA in cholesterol-fed rats, and \(\alpha\)-hydroxylase mRNA is induced.\(^4\),\(^18\)-\(^40\) Thus, our results not only illustrate differences in gene expression between the endogenous and exogenous forms of hypercholesterolemia in rabbits, but they also imply fundamental species differences with regard to regulation of gene expression by dietary cholesterol.

In summary, the potent and selective ACAT inhibitor CI-976 is efficacious in a rabbit model of EH. The combined data are consistent with the hypothesis that efficacy is due to inhibition of liver ACAT and subsequent secretion of relatively cholesterol ester-poor hepatic lipoproteins. Thus, the data lend further support to the suggestion,\(^7\),\(^8\) that the hypercholesterolemia in EH rabbits is due in part to hepatic lipoprotein overproduction, and it broadens the current view regarding the types of experimental conditions in which certain ACAT inhibitors are efficacious. Finally, the hepatic mRNA profiles illustrate that marked changes in LDL cholesterol can be attained pharmacologically without altering the apparent rates of hepatic gene transcription for proteins currently thought to be important in lipoprotein and cholesterol metabolism (eg, LDL receptor, \(\alpha\)-hydroxylase, and HMG-CoA reductase).

Acknowledgments

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References


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