Liver-Specific Inhibition of Acyl-Coenzyme A:Cholesterol Acyltransferase 2 With Antisense Oligonucleotides Limits Atherosclerosis Development in Apolipoprotein B100–Only Low-Density Lipoprotein Receptor−/− Mice

Thomas A. Bell III, J. Mark Brown, Mark J. Graham, Kristina M. Lemonidis, Rosanne M. Crooke, Lawrence L. Rudel

Objective—The purpose of this study was to determine the effects of liver-specific inhibition of acyl-coenzyme A:cholesterol acyltransferase 2 (ACAT2) on the development of hypercholesterolemia and atherosclerosis in mice.

Methods and Results—Apolipoprotein B100–only low-density lipoprotein (LDL) receptor−/− mice were given saline, a nontargeting control antisense oligonucleotide (ASO), or ASOs targeting ACAT2 biweekly for a period spanning 16 weeks. Mice treated with ACAT2 targeting ASOs had liver-specific reduction in ACAT2 mRNA, yet intestinal ACAT2 and cholesterol absorption was left undisturbed. ASO-mediated knockdown of ACAT2 resulted in reduction of total plasma cholesterol, increased levels of plasma triglyceride, and a shift in LDL cholesteryl ester (CE) fatty acid composition from mainly saturated and monounsaturated to polyunsaturated fatty acid enrichment. Furthermore, the liver-specific depletion of ACAT2 resulted in protection against diet-induced hypercholesterolemia and aortic CE deposition. This is the first demonstration that specific pharmacological inhibition of ACAT2, without affecting ACAT1, is atheroprotective.

Conclusions—Hepatic ACAT2 plays a critical role in driving the production of atherogenic lipoproteins, and therapeutic interventions, such as the ACAT2-specific ASOs used here, which reduce acyltransferase 2 (ACAT2) function in the liver without affecting ACAT1, may provide clinical benefit for cardiovascular disease prevention. (Arterioscler Thromb Vasc Biol. 2006;26:1814-1820.)

Key Words: cholesteryl esters ♦ lipoproteins ♦ atherosclerosis ♦ gene expression ♦ antisense

Atherosclerosis is a chronic disease of arterial degeneration that can ultimately lead to coronary heart disease, the leading cause of death in the United States. Many epidemiological and mechanistic studies have established that plasma cholesterol circulating on low-density lipoproteins (LDLs) is the primary risk factor for coronary heart disease. In response to these findings, many therapeutic agents have been developed that lower plasma cholesterol. The 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors or statins have been the most successful and widely used of these drugs. However, recent reports, like Adult Treatment Panel III, have shown that alternative treatments are needed in addition to statins, particularly in patients who experience severe hypercholesterolemia or have had a previous coronary event. Compounds that inhibit the ACAT enzymes have long been an attractive target for therapy because this enzyme catalyzes cholesterol esterification in tissues and plays a key role in intracellular cholesterol balance, dietary cholesterol absorption, and lipoprotein particle secretion.

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There are 2 isoforms of ACAT: ACAT1 and ACAT2. Each isoform has a distinct pattern of expression among tissues shared among mice, monkeys, and humans. ACAT2 is predominately found in the liver within hepatocytes and in the small intestine within enterocytes. In contrast, hepatocytes and enterocytes appear to exclude expression of ACAT1, which is ubiquitously expressed in most other tissues. Preliminary studies of the role of ACAT in atherosclerosis focused primarily on ACAT1 because of its expression in macrophages and its apparent role in foam cell formation within atherosclerotic plaques. Rather, toxicity apparently related to intracellular free cholesterol accumulation within several tissues was evident. Subsequent inflammation and macrophage apoptosis appear to have occurred as a result. In
contrast, ACAT2\(^{-/-}\) mice bred onto either an apolipoprotein E–deficient (apoE\(^{-/-}\)) or LDL receptor–deficient (LDLr\(^{-/-}\)) background were protected against atherosclerosis without any obvious signs of toxicity.\(^5\) Lipoprotein analysis in these mice showed a reduction in plasma cholesterol without a large effect on plasma apoB concentrations.\(^6\) The reduction of atherosclerosis after ACAT2 gene deletion was most likely related to the primary function of the enzyme in the generation of cholesteryl esters (CEs), mainly cholesteryl oleate in chylomicrons and in nascent very low–density lipoproteins (VLDL).

Many ACAT inhibitors have been developed, yet most of these compounds are not isoform specific (L.L. Rudel and S.L. Sturley, unpublished observation, 1999). Although studies have shown that many inhibitors can reduce plasma cholesterol and atherosclerosis in experimental animals, separation of effects of the inhibition of ACAT1 from the effects on ACAT2 in the liver and intestine has not been accomplished.\(^6\) In the present study, we used second-generation antisense oligonucleotides (ASOs) designed to specifically inhibit ACAT2 by enhancing ACAT2 mRNA degradation through an RNaseH–mediated degradation process.\(^1\) Based on the well-characterized pharmacokinetic properties of ASOs, these compounds should preferentially decrease hepatic ACAT2 without altering the activity of ACAT2 in the intestine or the availability of ACAT1 in many other tissues.\(^1\) This allows the unique opportunity to examine the role of hepatic cholesterol esterification by ACAT2 in promoting atherosclerosis.

Methods

Mice and Diet

The mice selected for this study were male apoB100 only, LDLr null mice that have a mixed background (\(
\sim75\%\) C57BL/6 and \(
\sim25\%\) 129Sv/Jae)\(^{20}\) and were originally obtained from Dr Steven Young (University of California, Los Angeles). To generate the control ACAT2\(^{-/-}\) mice used in this study, the apoB100 only, LDLr\(^{-/-}\) mice were crossed with ACAT2\(^{-/-}\) LDLr\(^{+/+}\) mice on a mixed background (50% C57BL/6 and 50% 129Sv/Jae)\(^{20}\) that had been created by Dr Robert Farese Jr (Gladstone Foundation, San Francisco, Calif.). From this mating, apoB100 only, LDLr\(^{-/-}\) mice were selected for further experimentation. The sequences of these ASOs are as follows: ASO 2: 5\('\)–GTGCCACTTCG-3\('\); ASO 3: 5\('\)–TGGAGAGCACCAAGACAGACA-3\('\); ASO control: 5\('\)–TGCCGGAGTCGACAATGAT-3\('\). ACAT2 forward 5\('\)–TGTCGCTAAACATCTGAATCC-3\('\); ASO 6: 5\('\)–GTGGAGAGCACCAAGACAGACA-3\('\); ASO control: 5\('\)–GTGCCACTTCG-3\('\). The ASO control is not complementary to the ACAT2 sequence and does not hybridize with any specific gene target. For all studies, mice were injected intraperitoneally with ASO (25 mg/kg) biweekly. One group of apoB100 only, LDLr\(^{-/-}\) mice received only the saline injections. The ACAT2\(^{-/-}\) mice were also injected with saline in a volume equivalent to the 25 mg/kg ASO dose (\(
\sim200\) \(\mu\)L).

ACAT2 ASO Tissue Specificity Study

For this study, mice were assigned to groups of 3 animals. Groups included ASO 2, ASO 6, and ASO control treatments for 4 weeks. Intestinal cholesterol absorption was measured by the dual fecal radioisotope method according to previously published methods.\(^2\) Values represent the percentage of cholesterol absorbed. At the end of the 4-week treatment period, the mice were fasted for 4 hours, euthanized, and plasma, liver, and intestine samples were taken and snap-frozen for subsequent analysis as described previously.\(^1\)\(^,\)\(^2\)

Atherosclerosis Study

For evaluation of atherosclerosis, groups of 11 to 13 mice were treated for 16 weeks. At 0, 4, and 8 weeks, the mice were restrained by administration of ketamine (50 mg/kg) and xylazine (10 mg/kg), and a blood sample was collected from the retroorbital plexus. At the end of the 16-week treatment period, the mice were fasted for 4 hours and overdosed with anesthetic. Plasma was isolated from blood collected from heart puncture in EDTA after centrifugation at 16 000X \(g\) at 4°C, and aliquots of plasma were stored in 10% percent sucrose at \(\sim20^\circ\)C. Liver and intestine samples were collected at the time of death, were snap-frozen in liquid nitrogen, and then stored at \(-80^\circ\)C. The aortas beginning at the aortic sinus and ending at the iliac bifurcation were removed from the mouse and fixed in a 10% neutral-buffered formalin solution.

Measurements to Detect Toxicity

After 16 weeks of treatment, 2 pooled plasma samples from each treatment group consisting of equivalent amounts of plasma from 3 mice were used for assessing hepatotoxicity. Enzyme activity in plasma for alanine transaminase (ALT), aspartate transaminase (AST), and bilirubin concentration were determined using a COBAS FARA II autoanalyzer using protocols and reagents supplied by Sigma Diagnostics.

Quantification of ACAT mRNA by Real-Time Polymerase Chain Reaction

The real-time polymerase chain reaction analyses of ACAT2 and ACAT1 mRNA in liver and small intestine were performed according to methods described previously.\(^2\) Primers used for these measurements were: ACAT1 forward 5\('\)–GGAGGATTTGGTGCACCTTCG-3\('\) and ACAT1 reverse 5\('\)–GGCTGCTTCAGATCTTTTG-3\('\). ACAT2 forward 5\('\)–GACTTGCGATCAATGGACTCG-3\('\) and ACAT2 reverse 5\('\)–GCTGCTTCAGATCTTTTG-3\('\). Cyclophilin forward 5\('\)–TGGAGACCCCAAGACAGACA-3\('\) and cyclophilin reverse 5\('\)–TGCGGCAGGTGCACAAATGAT-3\('\).

Plasma Lipid and Lipoprotein Measurements

Total plasma cholesterol (TPC), free cholesterol, and triglyceride (TG) concentrations were measured using enzymatic assays as described previously.\(^2\)\(^,\)\(^3\) The cholesterol distribution among lipoproteins was measured for plasma samples collected at the conclusion of the 16-week treatment intervention. Briefly, potassium bromide was used to adjust the solvent density of 0.5 mL of plasma to 1.225 g/L, and lipoproteins were isolated from the top of the tube after ultracentrifugation in a 100.2 rotor in a TL8 table-top ultracentrifuge operated at 80 000 rpm for 6 hours. The respective lipoprotein classes were then separated by chromatography on a Superose 6 gel-filtration column according to previously described methods.\(^4\) LDL particle size determination, estimated as LDL molecular weight in g/\(\mu\)mol, was measured by comparing column retention times of individual LDL samples with the retention times of LDL reference standards. The fatty acid composition of CE and TG from isolated chylomicrons and in nascent very low–density lipoproteins (VLDL).

The fatty acid composition of CE and TG from isolated chylomicrons and in nascent very low–density lipoproteins (VLDL).
LDL was determined by fatty acid methyl ester analysis as described previously.\textsuperscript{14,23} Briefly, the lipid of the LDL samples was extracted in chloroform and methanol, as described by Bligh and Dyer,\textsuperscript{24} and lipid classes were separated by thin layer chromatography. Bands corresponding to CE and TG were removed, saponified, and the fatty acids were then methylated. Fatty acyl methyl ester composition was determined by gas chromatography.

**Liver and Aorta CE Measurement**

Liver CE concentration was estimated after performing chloroform:methanol extraction on ∼100 mg of liver tissue according to the Bligh and Dyer method.\textsuperscript{24} An aliquot of lipid extract was then solubilized in 1% Triton X-100 solution, and total and free cholesterol content was determined by enzymatic assays as described previously.\textsuperscript{21,22} The amount of esterified cholesterol was found by subtracting free cholesterol from the total cholesterol, and the difference was then multiplied by 1.67 to convert it to CE mass.

The extent of aortic atherosclerosis was measured by quantifying the accumulation of CE in the aorta by previously described methods.\textsuperscript{25} Briefly, the formalin-preserved aortas were cleaned by removing all adherent adipose and connective tissue; lipids were extracted in 2:1 chloroform:methanol with 5x-cholestanol added as an internal standard, and free and total cholesterol (after saponification) was measured by gas-liquid chromatography.

**Results**

Pilot studies were performed in vitro and in vivo to evaluate the efficacy of candidate ASOs (data not shown). Initial screening was done in isolated murine hepatocytes, and only those ASOs effective in reducing ACAT2 mRNA were further evaluated. In a preliminary in vivo study of 2-week duration, 2 ASOs, termed ASO 2 and ASO 6, were found to be effective in reducing hepatic ACAT2 mRNA and protein. Dosing at 25 mg/kg and 50 mg/kg was evaluated, and the 25 mg/kg dose was selected for the long-term studies because it was essentially as effective as the higher dose.

Body weight was monitored throughout the study and is displayed in Figure 1A. The body weights for the control, saline-treated, and ASO 6–treated mice increased to ∼28 g and then remained constant throughout the remainder of the study. The mice administered ASO 2 showed the same pattern of body weight increase up to 8 weeks, but this group was significantly lower than other groups at 16 weeks. To test whether this drop in body weight in mice treated with ASO 2 was related to potential toxicity of this compound, plasma ALT, AST, and bilirubin levels were measured (data not shown). At 16 weeks, mice administered ASO 2 had 5- and 4-fold increases in ALT and AST but no change in bilirubin when compared with the other treatment groups, which were all equivalent for these measurements. After 8 weeks, the ACAT2 knockout mice showed a significantly higher body weight, and weights of these mice continued to increase for the remainder of the study. We performed a separate study to demonstrate that the weight difference was not related to ACAT2 but is a background strain difference.

TPC values were monitored in all mice throughout the 16-week atherosclerosis study, and the results for all treatment groups are displayed in Figure 1B. Differences were readily apparent after 4 weeks. The mice treated with either ASO 2 or ASO 6 and the ACAT2\textsuperscript{−/−} mice had comparable and significantly lower TPC concentrations when compared with the saline and ASO control groups, which were similar. These differences were maintained throughout the study, although the TPC for the saline-treated mice decreased unexpectedly at 16 weeks. The lower TPC of mice treated with ASO 2 at 16 weeks was confounded by the weight loss and increase in liver enzyme levels seen in this group. Whole plasma TG concentrations were also measured in the terminal plasma sample (Figure 1C). The values in the saline and ASO control mice were consistently in the 150 to 200 mg/dL range. In contrast, TG levels in mice receiving ASO 2 or ASO 6 were significantly higher (300 to 350 mg/dL), and plasma TG concentrations in ACAT2\textsuperscript{−/−} mice averaged >500 mg/dL. Hypertriglyceridemia is produced when ACAT2 inhibition leads to increased secretion of TGs in nascent VLDL particles by the liver through an as yet undefined mechanism. To assure that plasma lipid changes were not related to effects on cholesterol absorption, functional assays were used to show that the ACAT2-specific ASOs did not change the
In Figure 2, we compared percent cholesterol absorption in apoB100-only, LDLr−/− mice treated with either ACAT2-targeting ASOs (ASO 2, ASO 6) or a nontargeting ASO (ASO control). ApoB100-only, LDLr−/− ACAT2−/− mice were treated with saline as a control. Columns represent averages (±SEM) for 3 male mice per treatment group, and different letters indicate significant differences between treatment groups, with P<0.05 by Tukey honestly significant difference test.

The percentage of cholesterol absorption by the intestine (Figure 2). In comparison, ACAT2−/− mice had a significantly lower percentage of cholesterol absorption that was down from >50% to ~26%, as described previously.20,21

To further evaluate the specificity of the ACAT2 ASOs, a 4-week ASO treatment was conducted, and ACAT1 and ACAT2 mRNA levels were compared in the liver and small intestine (Figure 3). As observed in the pilot study, mice injected with either ASO 2 or ASO 6 displayed an ~80% decrease in hepatic ACAT2 mRNA levels when compared with mice treated with the control ASO (Figure 3A). Similar results were seen in hepatic ACAT2 protein expression (data not shown). As expected, neither ASO 2 nor ASO 6 had any effect on intestinal ACAT2 expression (Figure 3B). Measurement of ACAT2 mRNA expression in the liver and intestine of ACAT2−/− mice resulted in a signal that was only slightly above background levels (Figure 3A and 3B). In contrast, ASO 2 and ASO 6 had no effect on ACAT1 expression in the liver or intestine, as shown in Figure 3C and 3D. The lower ACAT1 mRNA abundance in the livers of the ACAT2−/− mice was unexpected (Figure 3C).

Aortic CE concentration was measured to quantify the extent of atherosclerosis within the aortae, and the results are shown in Figure 4A. The lowest concentration of CE was found in the aortae of ACAT2−/− mice, and the amount in the ASO 2 treatment group was similar (means of 3.1 and 3.4 mg/g wet weight, respectively). The value found for the ASO 6 treatment group (6.5 mg/g wet weight) was also significantly lower than those of the saline and ASO control groups (means of 10.3 and 11.4 mg/g wet weight, respectively). Figure 4B represents the liver CE values measured in all treatment groups. ACAT2−/− and the ASO 2 mice had the lowest amounts of hepatic CE (means of 1.9 and 2.2 mg/g wet weight, respectively). The liver CE concentration of the ASO 6 treated mice (3.8 mg/kg wet weight) was intermediate between the ASO 2, ACAT2−/−, and the ASO control mice. The ASO control treatment group had a liver CE value of 9.9 mg/g wet weight, which was significantly greater than the ASO 2 treatment group and the ACAT2 knockout mouse. The highest level of liver CE was found in the saline-treated group at 25.9 mg/g wet weight, a value that was significantly greater than the values reported for any of the other treatment groups.

To further investigate differences in atherosclerosis, lipoprotein cholesterol distribution was examined in the plasma taken after 16 weeks of diet induction (Table). The TPC concentrations reflect similar values to those seen in
Lipoprotein Cholesterol Distribution and LDL Size

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Cholesterol (mg/dL)</th>
<th>LDL Size (g/μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole Plasma</td>
<td>VLDL</td>
</tr>
<tr>
<td>ASO 2</td>
<td>385±70 (a)</td>
<td>109±7 (a)</td>
</tr>
<tr>
<td>ASO 6</td>
<td>698±44 (b)</td>
<td>125±25 (a)</td>
</tr>
<tr>
<td>ASO control</td>
<td>1047±120 (c)</td>
<td>171±17 (a)</td>
</tr>
<tr>
<td>Saline</td>
<td>744±14 (b,c)</td>
<td>146±8 (a)</td>
</tr>
<tr>
<td>ACAT2−/−</td>
<td>624±31 (a,b)</td>
<td>114±7 (a)</td>
</tr>
</tbody>
</table>

Figures represent mean±SEM for 4 male mice in each treatment group. Different letters indicate a significant difference, with P<0.05 by Tukey honestly significant difference test.

HDL indicates high-density lipoprotein.

Figure 1B. No statistically significant differences among treatment groups were found for VLDL cholesterol concentration. However, the trend appeared to be for higher VLDL cholesterol in mice treated with ASO control. The lowest amount of LDL cholesterol was present in the ASO 2 treatment group. The ACAT2−/− and the ASO 6 mice had similar LDL cholesterol concentrations that were substantially lower than those of the ASO control mice, whereas the saline treatment group had similar LDL cholesterol concentrations to the former 2 groups. This reflects the unexpected decrease in TPC seen at 16 weeks in the saline-treated group. There were no significant differences in high-density lipoprotein cholesterol among the different treatment groups, although an apparent trend for higher high-density lipoprotein cholesterol in the ACAT2−/− mice was suggested.

LDL particle size was measured in each of the treatment groups because this variable has been positively associated with hepatic secretion rates of ACAT2-derived CEs. The ACAT2−/− mice and mice treated with either of the ACAT2-specific ASOs had smaller LDL particles than mice in the saline and ASO control groups, as shown in the Table. The average LDL size in the mice treated with ASO 6 was 4.34 g/μmol, whereas LDLs in the ACAT2−/− mice were even smaller (3.87 g/μmol). The largest LDL particles were found in the ASO control and saline treatment groups at 5.01 and 5.77 g/μmol, respectively. The mice treated with ASO 2 had the smallest LDL particles at 3.36 g/μmol. The contribution of ACAT2-derived CEs is believed to contribute to the overall size enlargement of the LDL particles, so the LDL CE fatty acid composition was measured as summarized in Figure 5. The LDLs of the ACAT2-specific ASO-treated mice and the ACAT2−/− mice had significantly decreased percentages of saturated and monounsaturated CE fatty acids when compared with the ASO control and saline treatment groups (Figure 5A); concurrently, these groups had significantly higher percentages of polyunsaturated CE fatty acids, reflecting a higher contribution of lecithin cholesterol acyltransferase (LCAT)-derived CEs (Figure 5B). As controls, we measured the LDL TG fatty acid compositions, which are not influenced by ACAT2. No statistically significant differences were observed in the percentages of saturated, monounsaturated, and polyunsaturated LDL TG fatty acids among any of the treatment groups (Figure 5C and 5D).

Discussion

The major finding of this study was that ACAT2-specific ASOs selectively reduced hepatic ACAT2 activity, protein, and mRNA in apoB100-only LDLr−/− mice. However, in the intestine, the other major site of ACAT2 expression, antisense compounds had no effect on ACAT2 activity, protein, or mRNA. Therefore, this represents the first demonstration of a liver-specific ACAT2 inhibitor in mice. A 16-week study was performed in male mice fed a diet containing saturated fat and cholesterol to induce atherosclerosis. Treatment with the ACAT2-specific ASO significantly reduced hepatic CE accumulation (Figure 4B), reduced total plasma and LDL cholesterol (Figure 1B; Table), and shifted plasma CEs to a predominantly polyunsaturated form (Figure 5). Cumulatively, these changes produced a diminution in the severity of atherosclerosis, as quantified by the reduction in aortic CE concentration. Only very small amounts of CE occur in a normal aorta. Because the aorta cannot degrade cholesterol, esterified cholesterol has long been recognized as the primary lipid that accumulates after plasma lipoprotein infiltration, providing a sensitive marker for lesion development. With this end point, we are able to objectively quantify atherosclerotic lesion extent throughout the entire aorta, preventing confounding by regional differences.

Therefore, for the first time, this study demonstrated that selective inhibition of hepatic ACAT2 is sufficient to reduce the extent of aortic atherosclerosis that developed in these mice. The reduction in plaque progression was even more pronounced in these apoB100-only LDLr−/− mice when the ACAT2 gene was silenced (Figure 4A), but the data show that a selective pharmacological inhibition of hepatic ACAT2 is sufficient to produce an atheroprotective effect. We believe these findings are significant and suggest that future studies should be directed toward the development of human liver-specific ACAT2 inhibitors. Compounds like ezetimibe are already available to reduce intestinal cholesterol absorption, and although a general ACAT2 inhibitor might be expected to provide some of these same benefits, the suppression of ACAT2 in the liver would be expected to provide additional atheroprotective advantages. A recent human clinical trial using the compound paticimibe failed to show efficacy for reduction of athero-
sclerosis as measured by intravascular ultrasound imaging. However, there were no efficacy parameters reported that would indicate that dosage in this study was sufficient to alter ACAT2 so that little indication about ACAT2 as a drug target in humans was provided.

The antisense-mediated reduction in hepatic ACAT2 expression did not alter ACAT1 mRNA abundance in the liver or other tissues. Such a selective inhibition of ACAT2 is desirable because earlier studies on atherosclerosis in ACAT1 knockout mice showed massive accumulations of liver or other tissues. Such a selective inhibition of ACAT2 expression did not alter ACAT1 mRNA abundance in the drug target in humans was provided.

Figure 5 summarizes the effect on LDL–CE lipid composition and illustrates the shift from ACAT2-derived saturated and monounsaturated CEs to the polyunsaturated CEs synthesized by LCAT. The compensatory mechanism of LCAT to synthesize LDL CEs was noted in previous studies. In studies in which LDLr mice lacking ACAT2, LCAT, or ACAT2 and LCAT were compared, ACAT2 mice were protected from atherosclerosis, whereas the LCAT mice had increased atherosclerosis when compared with the control groups. The increase in atherosclerosis in the LCAT mice was attributed to an increase in the proportion of ACAT2-derived CE in LDL, indicating that the monounsaturated and saturated CE made by ACAT2 is proatherogenic.

The plasma lipid concentrations of the mice treated with the ACAT2 ASOs were analogous to those found in the ACAT2−/− mice, although the magnitude of change was greatest when the gene was genetically silenced. The action of LCAT to fill the depleted neutral lipid core of the LDL in these mice with CE is a compensatory factor. Figure 5 shows that the ACAT2 ASOs were analogous to those found in the ACAT2−/− mice, although the magnitude of change was greatest when the gene was genetically silenced. The action of LCAT to fill the depleted neutral lipid core of the LDL in these mice with CE is a compensatory factor. Figure 5 shows that the ACAT2 ASOs were analogous to those found in the ACAT2−/− mice, although the magnitude of change was greatest when the gene was genetically silenced. The action of LCAT to fill the depleted neutral lipid core of the LDL in these mice with CE is a compensatory factor. Figure 5 shows that the ACAT2 ASOs were analogous to those found in the ACAT2−/− mice, although the magnitude of change was greatest when the gene was genetically silenced. The action of LCAT to fill the depleted neutral lipid core of the LDL in these mice with CE is a compensatory factor. Figure 5 shows that the ACAT2 ASOs were analogous to those found in the ACAT2−/− mice, although the magnitude of change was greatest when the gene was genetically silenced. The action of LCAT to fill the depleted neutral lipid core of the LDL in these mice with CE is a compensatory factor. Figure 5 shows that the ACAT2 ASOs were analogous to those found in the ACAT2−/− mice, although the magnitude of change was greatest when the gene was genetically silenced. The action of LCAT to fill the depleted neutral lipid core of the LDL in these mice with CE is a compensatory factor. Figure 5 shows that the ACAT2 ASOs were analogous to those found in the ACAT2−/− mice, although the magnitude of change was greatest when the gene was genetically silenced. The action of LCAT to fill the depleted neutral lipid core of the LDL in these mice with CE is a compensatory factor.

The role of TG in atherosclerosis is still poorly understood. In our studies, as shown in Figure 4C, the ACAT2−/− mice and those treated with ACAT2-specific ASOs were protected from atherosclerosis despite having significantly higher amounts of plasma TG. Increases in plasma TG have often been found in mice in which ACAT2 activity has been deleted regardless of the diet. In the initial atherosclerosis study, in which ACAT2−/− mice were crossed with mice lacking apoE and fed chow for 27 weeks, a near doubling of plasma TG resulted. In another study in which ACAT2−/− mice on an LDLr background were fed an intact ACAT2 enzyme (Table), a situation in which atherosclerosis is more extensive (Figure 4A). In this case, we suspect LDL size is increased because of the proatherogenic ACAT2-derived saturated and monounsaturated CEs in the plasma LDL particles.

Bell et al Antisense ACAT2 Limits Atherosclerosis in Mice

![Figure 5. LDL CE (A and B) and TG fatty acid (C and D) composition of apoB100-only, LDLr−/− mice treated with ACAT2-targeting ASOs (ASO 2, ASO 6), a nontargeting ASO (ASO control), or saline.](http://atvb.ahajournals.org/)

<table>
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<th>LDL-CE Sat+Mono</th>
<th>LDL-CE Poly</th>
<th>LDL-TG Sat+Mono</th>
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<td>ASO 6</td>
<td>5</td>
<td>10</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>ASO C</td>
<td>7</td>
<td>12</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>ASO T2</td>
<td>8</td>
<td>15</td>
<td>16</td>
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*Columns represent averages (±SEM) for 3 male mice per treatment group, and different letters reflect significant differences with *P*<0.05 by Tukey honestly significant difference.
perfusion studies conducted in ACAT2−/− mice have revealed a higher rate of TG accumulation in perfusate, supporting the theory that the increase in plasma TG is attributable to a compensatory increase in TG secretion from the liver. Despite these ACAT2-associated alterations in TG metabolism, no study has shown a negative effect of such TG accumulation on atherosclerosis in ACAT2−/− mice.

Our studies have provided initial evidence that selective inhibition of hepatic ACAT2 by ASOs is possible and can be an effective means to reduce the progression of atherosclerosis. Similar studies need to be conducted in animal models whose overall physiology is more analogous to humans to further evaluate the potential of using such compounds as antiatherogenic agents in humans.

Disclosures

None.

References

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