The Nine Lives of ACAT Inhibitors

Robert V. Farese Jr

Atherosclerosis is the product of excessive lipid accumulation and inflammation in the artery wall. The lipid that tops every list of suspects is cholesterol, which is the primary lipid in low-density lipoproteins (LDL). Cholesterol exists either as a simple molecule or as cholesterol esters, in which the hydroxyl group is linked to a fatty acyl moiety. In cells, cholesterol esters are synthesized in an intracellular reaction catalyzed by acyl coenzyme A (CoA): cholesterol acyltransferase (ACAT) enzymes.\(^1\,\,!\,\,^2\) Owing to the discoveries of cholesterol esters in arterial lesions in 1910\(^3\) and of ACAT activity in the mid 1900s,\(^4\) inhibiting ACAT has been considered as a strategy for preventing or treating atherosclerosis. Over the past 25 years, interest in ACAT inhibitors has waxed and waned as new studies advance knowledge in the field. Prominently reported and disappointing results from a recent human trial of an ACAT inhibitor\(^5\) dampened enthusiasm for this potential therapy. However, a study by Bell et al in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*\(^6\) revives the idea of targeting ACAT enzymes and highlights a key unanswered question: Can ACAT2-specific inhibitors lower plasma cholesterol and treat or prevent atherosclerosis?

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After the discovery of the ACAT reaction, two rationales for inhibiting ACAT emerged. One, in retrospect, was perhaps overly simplistic: blocking cholesterol esterification in macrophages would diminish macrophage “foam cell” formation and thereby decrease atherosclerotic lesion development. The other was to decrease hepatic and intestinal cholesterol ester formation, resulting in decreases in plasma levels of the atherogenic apolipoprotein B–containing lipoproteins, such as cholesterol ester–rich LDL and remnant lipoproteins derived from very low-density lipoproteins and chylomicrons. With these ideas in mind, pharmaceutical companies initiated drug discovery programs, and several potent inhibitors were discovered.\(^1\) Between 1980 and 1995, interest in ACAT inhibitors grew steadily, as reflected by a gradual increase in articles published on the subject (peaking at \(\approx 30\) per year in 1995). However, studies in humans showed that several compounds lacked efficacy for lowering cholesterol,\(^7\) some compounds exhibited toxicity in animal studies,\(^8\)\(^10\) and statins emerged to take their predominant role in the treatment of hypercholesterolemia. As a result, the idea of ACAT inhibitors as useful drugs lost vitality.

In 1993, the ACAT field was resuscitated with the cloning of an ACAT gene,\(^11\) a heroic effort that took nearly a decade. ACAT investigation entered the molecular era, which afforded new approaches to elucidate the functions of the enzyme. Mice lacking the newly cloned gene were generated. These animals lacked cholesterol esters in macrophages and the adrenal cortex but had residual ACAT activity in the liver and small intestine,\(^12\,\,^13\) indicating that another ACAT gene existed. Three laboratories subsequently cloned a gene encoding ACAT2,\(^13\,\,^14\,\,^15\) which is expressed in the liver and small intestine. No other ACAT genes have been identified, suggesting that ACAT1 and ACAT2 are the only two ACAT enzymes in mammals.

The discovery of two ACAT enzymes raised the question of whether selective ACAT inhibition would lower plasma cholesterol levels or treat atherosclerosis. Subsequent studies in mice afforded some predictions. Mice lacking ACAT1 exhibited toxic accumulation of unesterified cholesterol in the skin and brain in the setting of hypercholesterolemia,\(^16\,\,^17\) and mice with macrophages engineered to lack ACAT1 had increased atherosclerotic lesions in a mouse model of atherosclerosis.\(^18\) These results suggested that the inability to esterify cholesterol in macrophages could result in cellular toxicity, giving rise to cell death and inflammation, a concept supported by studies using ACAT inhibitors in cultured macrophages.\(^19\)\(^20\) Thus, it was predicted that ACAT1 inhibition would not be a good strategy and, in fact, could have detrimental consequences.\(^1\)

In contrast, mice lacking ACAT2 exhibited attractive metabolic findings. These included a restricted capacity to absorb cholesterol and protection against diet-induced hypercholesterolemia and gallstone formation.\(^21\)\(^22\) Further, they lacked cholesterol esters in their apolipoprotein B–containing lipoproteins and were protected from atherosclerosis in murine models of the disease.\(^23\) Mice lacking both ACAT2 and lecithin:cholesterol acyltransferase (LCAT), an enzyme that catalyzes cholesterol ester synthesis in the plasma lipoproteins, had virtually no cholesterol esters in the plasma and were highly resistant to atherosclerosis,\(^24\) underscoring the importance of plasma cholesterol esters in atherosclerosis. These studies in rodents raised the important question of whether ACAT2-specific inhibition would protect against atherosclerosis.

In the past few years, enthusiasm for ACAT inhibitors again has again waxed and waned. Several studies in animals, primarily using nonselective inhibitors, showed promising results.\(^25\)\(^26\)\(^27\) However, two recent trials in humans were disappointing. In one, the nonselective inhibitor avasimibe, administered for 2 years to patients with atherosclerosis, did

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*Arterioscler Thromb Vasc Biol.* 2006;26:1684-1686.

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*Arterioscler Thromb Vasc Biol.* is available at http://www.atahaha.org

DOI: 10.1161/01.ATV.0000227511.35456.90

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not reduce plaque volume. More recently, an 18-month study of patients with atherosclerosis treated with pactimibe also failed to show a reduction of plaque volume, and secondary plaque-related end points suggested a detrimental effect of the drug. However, there are caveats to interpreting the pactimibe study. First, the drug is reportedly nonselective. Second, no evidence suggested that therapeutic efficacy was achieved with respect to ACAT2 inhibition; plasma cholesterol levels were unaffected, suggesting that inhibition of liver and intestinal ACAT activity was insufficient. Nevertheless, the latter trial prompted the authors to pronounce the imminent death of ACAT inhibitors as a viable atherosclerosis therapy.

The study of Bell et al in this issue breathes life back into the idea of ACAT2-specific inhibition. In atherosclerosis-prone mice, ACAT2 was specifically inhibited in the liver with antisense oligonucleotides. Biweekly intraperitoneal injections, which reduced ACAT2 expression by a remarkable 80%, decreased diet-induced hypercholesterolemia and sharply reduced cholesterol ester deposition in the aorta. It also reduced the levels of saturated and monounsaturated fatty acids in cholesterol esters in plasma LDL and increased the levels of polyunsaturated fatty acids. The latter findings reflect the increased relative contribution from LCAT. The antisense treatment did not affect ACAT2 expression in the small intestine, nor did it affect intestinal cholesterol absorption.

The antisense approach is intriguing, particularly given the success of intermittent injections of antisense oligonucleotides to lower plasma apoB and cholesterol levels. The prolonged and marked knockdown of hepatic gene expression from a single dose of antisense oligonucleotides is impressive. However, one of the ACAT2 antisense oligonucleotides in the study of Bell et al was associated with liver toxicity, indicating the need for caution as antisense approaches move into human trials.

A fundamental question remains: would ACAT2-specific inhibition in humans, either pharmacologically or with antisense oligonucleotides, prevent or reduce atherosclerosis? A key factor may be whether ACAT2 activity is as important in human liver as it is in mouse liver. ACAT activity in human liver is lower than in other species, and it is controversial whether ACAT is the predominant form in human hepatocytes. Another consideration is the relative contribution of ACAT enzymes to plasma cholesterol esters in humans. As noted above, cholesterol esters are also formed in plasma lipoproteins through a reaction catalyzed by LCAT, which contributes substantially to circulating cholesterol esters. Finally, ACAT2 expression has been reported to be upregulated in macrophages of atherosclerotic lesions, which current evidence suggests is not a good place for ACAT inhibition.

Nevertheless, the findings of Bell et al suggest that ACAT2-specific inhibition needs to be tested. As the great physicist Richard Feynman liked to remind us, “The sole test of the validity of an idea is experiment.” Until a potent and specific inhibitor of ACAT2 is tested in humans, the hypothesis remains untested. Meanwhile, the idea of using ACAT inhibitors to treat atherosclerosis is again moribund, awaiting an experiment to determine its fate.

Disclosures

None.

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Arterioscler Thromb Vasc Biol. 2006;26:1684-1686
doi: 10.1161/01.ATV.0000227511.35456.90

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