Acyl-coenzyme A:cholesterol acyltransferases

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Cholesterol is a lipid molecule present in the membranes of all mammalian cells and is needed for their growth and viability. Excess cellular cholesterol is stored as cholesteryl esters. The conversion of cholesterol to cholesteryl esters (CE) is catalyzed by the enzyme acyl-coenzyme A (CoA):cholesterol acyltransferase (ACAT) (Fig. 1). In most cell types, CE are present only in low levels, mainly as cytoplasmic lipid droplets. In plasma, CE are part of the neutral lipid cargo present in the intestinal chylomicrons and in the hepatic very low-density lipoproteins (VLDL). In steroidogenic tissues such as adrenals, CE serve as the cholesterol reservoir for producing steroid hormones. In the disease atherosclerosis, chronic accumulation of CE in macrophages causes these cells to appear foamy and is a hallmark of early stages in atherosclerosis. For reasons described above, ACAT has been considered as a drug target for therapeutic intervention against atherosclerosis and other human diseases (Fig. 1).

ACAT Genes and Proteins

Gene ACAT1 and its mRNAs. The first ACAT gene was identified in 1993 (16). The ACAT family consists of ACAT1, ACAT2 (3, 10, 67, 93, 100), and acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) (11). These enzymes are founding members of the membrane-bound O-acyltransferase (MBOAT) enzyme family. MBOATs are multispan membrane enzymes that utilize long-chain or medium-chain fatty acyl-CoA and a hydrophobic substance as their substrates (39). These enzymes contain an invariant histidine (H) within a long stretch of hydrophobic residues and a near-invariant asparagine (N) within a stretch of modestly hydrophilic residues (39, 94). In addition to ACAT and DGAT1, the additional MBOATs include grelin octanoyl-CoA acyltransferase (38, 94) and lysophospholipid acyltransferases (reviewed in Ref. 81). Human ACAT1 (hACAT1) is located on two different chromosomes, chromosomes 1 and 7, with each chromosome containing a distinct promoter: P1, contiguous with exons 1–16, is located in chromosome 1; and P7, contiguous with the optional long exon Xa, is located in chromosome 7 (55). This novel arrangement is almost unprecedented in the mammalian genome and is species specific. In mice, although the Acat1 gene is also located in chromosome 1, it does not contain the optional exon Xa present in hACAT1. The gene Acat1 is not under the control of the transcription factors sterol regulatory element-binding proteins, which control the expression of many genes involved in cellular lipid metabolism (33). In response to cellular cholesterol depletion/repletion, the alteration of ACAT1 activity occurs within 1–2 h (15), i.e., at a time frame much faster than what is needed for transcriptional control mechanism(s) to be in full operation.

There are at least three human Acat1 mRNAs. The 2.8- and 3.6-knt Acat1 mRNAs contain a short 5′-untranslated region (UTR) and comprise most (70–80%) of the total Acat1 mRNAs that are produced from the hACAT1 P1 promoter. These mRNAs are translated as a single 50-kDa protein band in SDS-PAGE, and the resultant protein is the major ACAT1 isoenzyme observed in various human cells and tissues (14). The 4.3-knt Acat1 mRNA instead contains the alternate long 5′-UTR. This mRNA is produced from two different chromosomes by a novel RNA recombination event. The 5′-UTR contains an alternative initiation codon and an extended in-frame sequence, enabling the chimeric mRNA to produce a larger ACAT1 isoform. In SDS-PAGE, it is detectable as a 56-kDa protein. In human macrophages, the 56-kDa protein is present as a minor ACAT protein (96). The physiological function of the 56-kDa ACAT1 is currently unknown.

In macrophage-like cells, the expression of hACAT1 is up-regulated by interferon-γ, a cytokine that exerts many proatherosclerotic effects in model animals (25, 95). Urotensin II, the potent vasoconstrictor peptide that can lead to hypertension and atherosclerosis in humans, upregulates hACAT1 expression in...
ACAT

Roles of ACAT in vivo

Figure 1. Roles of acyl-CoA:cholesterol acyltransferase (ACAT) in vivo (courtesy of Prof. Akira Miyazaki, Showa University). CE, cholesteryl ester; CoASH, free reduced CoA.

human macrophages (89). In addition, the synthetic glucocorticoid dexamethasone, known to promote atherosclerosis in humans (47), upregulates hAcat1 expression in macrophages. Consistent with this observation, a glucocorticoid response element is present within the hAcat1 P1 promoter (97). In contrast, hAcat1 expression is downregulated by adiponectin, an adipocytokine that exerts many antiatherosclerotic effects in cell culture studies (32). Together, these results support the concept that upregulation of Acat1 expression in monocytes and macrophages is closely associated with the initiation and progression of the atherosclerosis in tissue culture and in experimental animal models and perhaps in humans.

Biochemical and cellular properties of ACAT1. Native ACAT1 is an integral membrane protein present in minute quantities in the endoplasmic reticulum (ER). The recombinant 50-kDa human ACAT1 overexpressed in Chinese hamster ovary (CHO) cells has been purified to homogeneity, with retention of catalytic activity (17). The enzyme responds to cholesterol as its substrate in a sigmoidal manner (17). Additional kinetic evidence has suggested the presence of a sterol substrate site and a sterol activator site in ACAT1. Cholesterol is superior to oxysterols and to any other sterol as an activator for ACAT1. Upon activation by cholesterol, ACAT1 activity is much increased, and the enzyme is promiscuous toward a variety of sterols with a 3-β-OH configuration in the B ring as its substrates (60, 102). These results demonstrate that ACAT1 is allosterically activated by cholesterol.

ACAT1 exists as a homotetrameric enzyme in intact cells and in vitro (99). Deleting the dimer-forming motif near the NH2 terminus converts the enzyme to a fully functionally active dimeric form (101). An additional motif(s) involved in forming the other dimer is located near the COOH terminus of the enzyme (35). ACAT1 contains one disulfide linkage and seven free cysteines (Cys). Guo et al. (36) employed the membrane-impermeable, sulfhydryl-specific reagent polyethylene glycol5000-maleimide (PEG5000-mal) to map the disulfide linkage and to probe the environment of the free sulfhydrys of the enzyme. Their results showed that the first free Cys is located near the NH2 terminus in the cytoplasmic side of the ER membrane. The other six free Cys are buried within several transmembrane domains (TMD) of the protein (36). None of the free Cys is required for the ACAT1 enzyme activity (62); the disulfide linkage is important for structural stability of the enzyme (36). Regarding the ACAT1 topography, initially, Lin et al. (58) used the method of epitope tagging and indirect immunofluorescence to fix intact cells. Their results suggested that ACAT1 might contain at least 7 TMDs; the region at the COOH-terminal half is very hydrophobic and might contain additional membrane imbedded segment(s). More recently, Guo et al. (37) used PEG5000-mal together with Cys-scanning mutagenesis to further investigate the ACAT1 membrane topography at the COOH-terminal half and proposed a revised nine-TMD model. The active site His460 is located in a hydrophobic environment, within a newly disclosed TMD (TMD no. 7), between R443-Y462 (Fig. 2). In contrast to the nine-TMD model, Joyce et al. (46) had proposed a five-TMD model for ACAT1. The five-TMD model was deduced by monitoring the sidedness of the tag at the end of the COOH terminus of the protein after successive deletions from the ACAT1 COOH terminus to produce various truncated ACAT1s. Similarly to the nine-TMD model, the five-TMD model also proposes the existence of the first four TMDs near the NH2-terminal half and the last TMD near the COOH terminus. However, the five-TMD model predicts that no other TMD exists and that all of the following Cys are located at the cytoplasmic side of the ER membrane: C333, C345, C365, C387, and C467. This prediction is incompatible with the data by Guo et al. (36), who showed that all of these Cys are resistant to modification by the membrane-impermeable reagent PEG5000-mal. To explain the discrepancy in ACAT1 membrane topography determination, a clue has been suggested (37); it is possible that the method of successive truncation(s) from the COOH terminus of ACAT1 employed by Joyce et al. (46) might have created major structural alteration(s) within the ACAT1 polypeptide. In support of this suggestion, Guo et al. (37) have indicated that, in their experience, even the modest truncation(s) from the COOH terminus of ACAT1 led to total inactivation of ACAT1 enzyme activity.

TMD7 (amino acids 443-461) and TMD8 (amino acids 463-483) of ACAT1 are rich in coiled domains and have two distinct functional sides. One side is involved in subunit interaction (to form an ACAT1 dimer); the other side of the coils, including the conserved F453, A457, H460, and F479, is involved in substrate binding and/or in catalysis. Residues F453, A457, and F479 may be part of a lipid-binding tunnel that interacts with the hydrophobic part of cholesterol, whereas residue H460 may interact with the 3-β-OH moiety of the sterol and serve as a general base in catalysis (35). It has been suggested that having the active site of ACAT1 located within the plane of the ER membrane allows the enzyme to serve a dual function; CE generated within the lipid bilayer can leave the cytoplasmic leaflet of the membrane to form lipid droplets or be recruited to the lumen of the membrane for the VLDL assembly process (21). Kinetic evidence from Guo et al. (34) suggests that the highly conserved residue FYXDWN (403-409 in hAcat1) may be involved in binding fatty acyl-CoA. Recently, Das et al. (23) showed that, in addition to H460, S456 and D400 are also essential for ACAT1 enzyme activity. These authors suggested that ACAT1 may contain thioesterase activity, and this activity may involve the classical Ser/Asp/His
catalytic triad, similar to the serine-dependent hydrolases. The results described above were all based on site-specific mutagenesis studies. For a given enzyme, a protein structural model is needed to carry out functional analysis at the refined biochemical level. However, at present, the crystal structure of ACAT1 or any related family member is not available.

Regulation and properties of ACAT2. The second mammalian ACAT gene, designated as Acat2, was identified through its homology to Acat1 (3, 10, 67). The hAcat2 mRNA encodes a single 46-kDa protein on SDS-PAGE. ACAT2 shares high homology with ACAT1 near the COOH terminus but not near the NH2 terminus. The enzymological properties of the two proteins are similar but not identical (2, 18, 60). hAcat2 promoter contains cis elements for the transcription factors Cdx2 and HNF1α. Both Cdx2 and HNF1α are required to stimulate the expression of hAcat2 (84). HNF1α is functionally expressed in a variety of tissues, including hepatocytes, pancreas, kidney, stomach, and intestinal epithelium, whereas Cdx2 is an intestine-specific transcription factor. Under normal conditions, high expression of Cdx2 is restricted to the differentiated cells of the intestinal villi. In adult livers, in human hepatocytes, and in the human liver cell line L02, the Cdx2 mRNA level is very low, and the amount of ACAT2 mRNA was <10% that of the ACAT1 mRNA. In contrast, various human hepatocellular carcinoma tumor cells contain elevated Cdx2. Notably, in the hepatoma cell line HepG2, due to elevated Cdx2 expression, the amount of ACAT2 mRNA is comparable with that of the ACAT1 mRNA (84).

ACAT2 is also an integral membrane protein. However, its membrane topology significantly differs from that of ACAT1. Regarding the ACAT2 topology, Lin et al. (59) inserted two different tags at various hydrophilic regions and expressed the recombinant proteins in mutant CHO cells lacking endogenous ACAT. Each tagged ACAT2 was expressed in the ER and was shown to be at least partially enzymatically active. They then used double cytoimmunofluorescence and protease protection assays to monitor the sidedness of the tags along the ER membranes (59). Their results suggest that ACAT2 may contain only two detectable TMDs but may also contain several additional membrane domains that are only partially embedded in the lipid bilayer. The conserved H434, equivalent to H460 in hACAT1, may be buried in the cytoplasmic side of the ER membrane (Fig. 3). In contrast to the two-TMD model, Joyce et al. (46) reported that ACAT2 contained five TMDs. They prepared a series of the recombinant ACAT2 proteins successively truncated from the COOH termini after each of the predicted TMDs; the topography was determined by monitoring the membrane sidedness of tags at the COOH termini of each truncated fusion protein. Both the insertion approach used by Lin et al. (59) and the truncation
approach used by Joyce et al. (46) run the risk of altering the membrane topography of ACAT2. Lin et al. (59) did report that each tagged ACAT2 employed was at least partially active enzymatically, whereas Joyce et al. (46) did not report the percentage of ACAT activity remaining in any of the truncated proteins employed. In the future, to resolve the discrepancy, other methods for membrane topography determination that produce minimal structural perturbations of ACAT2 will be needed. However, a major discrepancy that cannot be explained by differential structural perturbation(s) of ACAT2 exists between the result of Lin et al. (59) and the result of Joyce et al. (46). Lin et al. (59) showed that the COOH terminus is in the cytoplasmic side of the ER, whereas Joyce et al. (46) showed that the COOH terminus is in the luminal side of the ER. To determine the location of the COOH terminus, both groups expressed the tagged protein by transient transfection in the same host cell line and placed certain tags near or at the COOH terminus of the protein. Both groups expressed the tagged protein by transient transfection in the same host cell line and then used cytoimmunofluorescence to examine the sidedness of the tag after detergent permeabilization. The results obtained sharply contradicted each other. Lin et al. (59) used the double immunostaining procedure for cytoimmunofluorescence and made sure that the two signals colocalized when viewed under microscopy. In contrast, Joyce et al. (46) did not use the double immunostaining procedure for their studies. Lin et al. (59) offered a clue that might explain the result obtained by Joyce et al. (46). The transfection procedure (used by both groups) tends to produce leaky and/or dying cells; such cells can amount to ≤5% of the total cell population and tend to be preferentially stained (nonspecifically) by antibodies. When cytoimmunofluorescence is employed in transiently transfected cells, the use of double immunofluorescence is essential; it enables the investigators to focus on viewing the transfected cells that express the gene product and to avoid the use of leaky/dying cells for data collection. Other explanations are also possible.

The relative expression of ACAT1 and ACAT2 in humans and in animals. In adult humans, studies carried out by Lee et al. (51) and by Chang et al. (13) using a combination of immunological staining, immunodepletion, and immunoblot analyses showed that ACAT1 is expressed in many different tissues and cell types, including hepatocytes and Kupffer cells of the liver, adrenal glands, neurons, and macrophages, and accounts for >80% of the total ACAT enzyme activity measured in vitro (18, 51, 80). In a separate study, Parini et al. (69) performed similar immunoblot analyses and demonstrated the presence of both ACAT1 and ACAT2 in adult human hepatocytes. These investigators then examined the ACAT enzyme activities in vitro in liver biopsy samples and showed that, among individual samples, the ACAT2 activities were highly variable, whereas the ACAT1 activities were relatively constant. Under the enzyme assay condition employed by these investigators, in three of the four liver biopsy samples examined, ACAT2 provided >50% of the total cholesterol-esterifying activity (69). It should be noted that the biopsy samples employed by these investigators were all collected from patients affected with gallstone disease; none was from a healthy subject. In another study, Smith et al. (83) performed real-time PCR analyses of Acat1 and Acat2 and showed that the ACAT1 mRNAs were on average ninefold (range of 1.7- to 167-fold) more abundant than the ACAT2 mRNAs. Among the 17
individual liver samples examined, the ACAT1 mRNA levels were relatively constant, whereas the ACAT2 mRNA levels were much more heterogeneous. From these studies, it is clear that both ACAT1 and ACAT2 are present in human hepatocytes, although the quantitative aspects of results were different. Together, these studies suggest that the expression of ACAT2 might be inducible under various conditions, whereas the expression of ACAT1 tended to be constitutive. This possibility is supported by the findings that, in activated human macrophages and in advanced atherosclerotic plaques, low but significant amounts of ACAT2 mRNA and protein are detectable (79). In addition, the liver samples of certain patients affected with hepatocellular carcinoma contain highly elevated ACAT2 mRNAs (84). It is also possible that subpopulations of humans may express hepatic ACAT2 at elevated levels constitutively. Further investigations are needed to test this possibility.

In intestines, ACAT2 is shown to be the major isoenzyme and is expressed mainly in the apices of the intestinal villi (13, 51); its mRNA in duodenum is approximately threefold more abundant than that of ACAT1 (83).

In mice and in monkeys, the ACAT1/ACAT2 distribution in various tissues is similar to that in humans except for at least one important difference, that ACAT2 is highly expressed in the livers of mice and monkeys under normal conditions (53, 64, 69). Available data show that in rabbits and mice the total ACAT enzyme activities in vitro (ACAT1 and ACAT2) are much higher than those in the human livers (discussed in Ref. 18). In African green monkeys, the hepatic ACAT2 activities are at least fourfold higher than those found in humans, whereas the hepatic ACAT1 activities in these two species are very similar (69). Regarding the roles of ACAT1 and ACAT2 in hepatic lipoprotein synthesis and secretion, in various model systems examined, both ACAT1 and ACAT2 are capable of synthesizing CE that contribute to the lipid core in VLDL (45, 56, 85). In mouse hepatocytes, ACAT2 is the major isof orm and plays a key role in the hepatic storage and packaging of CE into apoB-containing lipoproteins (6, 8, 52, 90). In human hepatocytes, both ACAT1 and ACAT2 are present. Whether ACAT1 and/or ACAT2 participate in the biosynthesis of CE in VLDL in intact human livers is yet to be determined. In mouse intestines, ACAT2 plays a key role in providing CE to chylomicrons (8, 72). Whether the same situation holds true in humans has not been determined.

**Acat1 knockout and Acat2 knockout mice.** Farese (28) created and characterized both the Acat1<sup>−/−</sup> and Acat2<sup>−/−</sup> mice. These mice have served as valuable research tools in lipoprotein and atherosclerosis research (reviewed in Ref. 9).

**ACAT and cellular cholesterol trafficking.** Cells acquire cholesterol mainly via two sources, from the low-density lipoprotein (LDL) and from de novo biosynthesis. Cholesterol molecules from both sources move within the cells, eventually reaching the ER; certain portions of them are converted to CE. In most of the cell types examined, ACAT1 plays the major role in catalyzing the reesterification process. The process of cellular cholesterol trafficking has been reviewed in Chang et al. (19), with more recent studies cited in Urano et al. (87).

**ACATs as Drug Targets**

**General properties of the ACAT inhibitors.** During the last several decades, numerous ACAT inhibitors have been synthesized and tested in test tubes, intact cells, animal models, and humans. We refer to two earlier reviews that summarize the structural characteristics and properties of the ACAT inhibitors (48, 82). A few of them are structural analogs of the long-chain fatty acyl-CoA. Almost all of the ACAT inhibitors are very hydrophobic compounds (40). The hydrophobic nature of these compounds may allow them to enter the membrane lipid bilayer such that they can interact with residues that play an essential role(s) in substrate binding and/or in enzyme catalysis. Interestingly, several natural substances present in the ordinary foodstuffs, such as esculeogenin A in tomatoes (31) and piperine in peppers (63), exhibit significant ACAT inhibitory activities. In addition, a peptide segment within the apo-serum amyloid A isoform 2.1 inhibits ACAT activity in macrophages (26).

**The isotype-specific ACAT inhibitors.** Most of the ACAT inhibitors developed in the 1990s inhibit both ACAT1 and ACAT2 without much selectivity. After the ACAT genes were identified, investigators began to develop ACAT isotype-selective inhibitors (22, 50, 68). One of the most notable examples is pyripyropene A, which prefers to inhibit ACAT2 over ACAT1 by >1,000-fold (68). Notably, glutamine (Q) 492, valine (V) 493, and serine (S) 494 of ACAT2 were shown to play an important role(s) for mediating the pyripyropene A inhibition (24). (The numberings of these amino acids are according to the monkey ACAT2 sequence.) The QVS sequence is part of the ACAT2 COOH-terminal segment. According to the model by Lin et al. (59), the COOH-terminal segment is located at the cytoplasmic side of the ER membrane; in contrast, according to the model by Joyce et al. (46), the COOH-terminal segment is located at the luminal side of the ER membrane. The QVS sequence is unique in ACAT2; its counterpart cannot be identified in ACAT1. A second notable ACAT isozyme-selective inhibitor called K 604 was developed by Kowa. K 604 prefers to inhibit ACAT1 over ACAT2 by >100-fold (43). The molecular basis that allows the preferential action of K 604 on ACAT1 is yet to be determined.

**The pros and cons of the ACAT inhibitors to combat atherosclerosis.** Whether ACAT inhibitors will serve as effective antiatherosclerosis drugs is currently under debate (see Refs. 12, 54, 66, 76, and 77 for different opinions). This review focuses on the effects of inhibiting ACAT in macrophages, aortic smooth muscle cells, and hepatocytes. The formation of CE-laden foam cells in the intimal layer of the arterial wall is an early event in atherogenesis (75). In humans, clinical observations suggest that the lipid-rich plaques are more prone to plaque rupture than the lipid-poor plaques (27, 57). Lipid-rich plaques consist of macrophages and smooth muscle cells (75). In foam cells, ACAT1 is the major isoenzyme (65). Cell culture studies suggest that foam cell formation in smooth muscle cells also depends on ACAT1 (65, 74). In model animals, administration of the isotype-nonselective ACAT inhibitor CI 1011 (5) or F-1394 (49) at low concentration, or the ACAT1-selective inhibitor K 604 (43), reduced the CE content within the atherosclerotic lesions and caused plaque regression without altering the overall serum cholesterol levels. In addition, in a rabbit model for atherosclerosis, the combination of
statin and CI 1011 synergistically reduced and stabilized the atherosclerotic plaques (92). However, when tested in human patients, CI 1011 was ineffective in potentiating the effect of statins to decrease the progression of coronary atherosclerosis (66). The latter result cannot be interpreted definitively because it is clouded by drug-drug interaction. CI 1011 has been shown to induce one of the cytochrome P450 enzymes, called CYP 3A4, in human hepatocytes through activation of the pregnane X receptor (78); CYP 3A4 could accelerate the catabolism of statins in vivo. Thus, the coadministration of CI 1011 and statin might allow CI 1011 to diminish the effective concentration of statin in vivo and weaken its effect.

The results of a mouse genetic approach showed that complete deletion of ACAT1 gene in macrophages actually increases the lesion size of the plaques (1, 29, 86). The cellular nature of the lesions of ACAT1-knockout mice is abnormal; these lesions are poor in macrophages and abundant in smooth muscle cells. The outcome of the ACAT1-knockout study implies that an overdose of ACAT1 inhibitor may cause the macrophages to become incompetent in fulfilling their functions as scavenger cells. Other results that disfavor the use of ACAT inhibitor as drug therapy for atherosclerosis include the finding that, when rodent macrophage cell lines were grown under conditions with minimal cellular cholesterol efflux, addition of ACAT inhibitor to the cells caused undesirable buildup of cellular free (unesterified) cholesterol, rendering the cells apoptotic (30, 88). These studies suggest that ACAT inhibitors can be highly toxic to macrophage cells present in the advanced atherosclerotic lesions. It should be pointed out that the cholesterol-dependent apoptosis observed in mouse macrophages cannot be observed in human macrophages (73). In addition, in contrast to the results in rodent macrophages, Rong et al. (74) showed that ACAT1 inhibition in rat and human aortic smooth muscle cells is nontoxic and retards foam cell formation. The discrepancy in results described above might be due to difference in the cell systems employed.

Regarding the effects of inhibiting ACAT in the liver, in animals (rodents, rabbits, and monkeys), ACAT2 is the major isoform. In various animal models tested, the ACAT isotype nonselective inhibitor CI 1011 (alone, without statin) significantly reduced serum cholesterol levels (reviewed in Ref. 61). In addition, deletion of ACAT2 gene in mice results in resistance to diet-induced hypercholesterolemia and to gallstone formation (8, 72), and it also results in protection against atherosclerosis (52, 90). Another study showed that, in a hyperlipidemic mouse model (apoB100 only, LDLr−/−), the in vivo administration of liver-specific antisense oligos against the ACAT2 mRNA reduced ACAT2 protein content only in the liver but not in the intestines; it also successfully reduced serum cholesterol level (4). The latter result suggests that blocking ACAT2 activity in the liver can significantly lower serum cholesterol levels and reduce atherosclerosis in a manner independent of dietary cholesterol absorption. On the other hand, when tested in human trials, administration of the ACAT inhibitor CI 1011 reduced VLDL cholesterol and triglyceride levels but failed to significantly reduce the LDL cholesterol levels (44, 61). These results suggest that the effects of CI 1011 on serum cholesterol levels are species dependent. To provide a possible explanation for these observations, as discussed earlier, the overall ACAT enzyme activity in human livers is much lower than that in rodents, rabbits, and monkeys (20, 69). Thus, hepatic ACAT in humans may not contribute to the CE content present in LDL as much as it does in experimental animal models. The expression of hepatic ACAT2 in humans seems to be highly heterogeneous; inhibiting ACAT2 may effectively reduce the CE content in LDL in subpopulations with elevated hepatic ACAT2. This possibility could be tested in the future.

ACAT inhibitors and Alzheimer’s disease. Alzheimer’s disease is the most prevalent neurodegenerative disease in developed countries. It is characterized by massive extracellular accumulation of amyloid plaques, composed mainly of amyloid-β (A-β) peptide aggregates, and intracellular accumulation of hyperphosphorylated tau protein in the cerebellar area. These events lead to widespread neurodegeneration in the brain. Age and genetic variation in apolipoprotein E, a plasma cholesterol transport protein, are two of the most important risk factors for Alzheimer’s disease (71, 98). In addition, studies suggest that cholesterol in membranes impacts on production of A-β (reviewed in Ref. 91). Amyloid precursor protein (APP) can be cleaved via two competing pathways, the α- and the β-secretase pathways, that are distinguished by the subcellular site of proteolysis and the site of cleavage within APP. Under normal conditions, several proteases are capable of catalyzing the α-cleavage, after which the γ-secretase complex, which includes presenilin as a catalytic subunit, further cleaves the APP fragment to produce small nonamyloidogenic fragments. Similarly, but competing with the α-cleavage pathway, the β-secretase pathway involves sequential cleavages by the β-secretase and γ-secretase complexes, which generates A-β. A-β is secreted from the cell to the extracellular space, where it aggregates over time. In the central nervous system, neurons are the major sources of A-β production. Evidence suggests that the activities of α-secretase, β-secretase, and γ-secretase are dependent on cholesterol metabolism. In CHO cells and various neuron-like cells grown in culture, reduction of CE either by genetic inactivation of Acat1 or by pharmacological inhibition of ACAT decreases A-β secretion (70). In addition, in a mouse model of Alzheimer’s disease, ACAT inhibitors CP-113,818 and CI 1011 substantially diminishes amyloid plaque density (41, 42). Although the exact mechanism by which ACAT inhibitors reduce amyloid plaques remains to be explored, these exciting findings suggest that ACAT inhibitors may serve as effective therapeutic or chemopreventive agents for a certain form(s) of Alzheimer’s disease.

Summary

ACAT1 and ACAT2 play key roles in cellular cholesterol metabolism. Understanding the functions of the ACAT enzymes has greatly increased the knowledge pertaining to cellular cholesterol homeostasis. Abnormalities in lipoprotein/cholesterol metabolism may lead to atherosclerosis. Although the statin drugs have significantly reduced the mortality of patients suffering from coronary heart disease, the risk of death among those with coronary heart disease remains high. As discussed in this chapter, inhibiting ACAT1 and ACAT2 may complement the action of statins by directly or indirectly diminishing foamy macrophage formation, thus further reducing the incidence of atherosclerotic cardiovascular disease. More recent evidence suggests that inhibiting ACAT enzyme activity may also ameliorate the symptoms of Alzheimer’s
disease. Further development of ACAT inhibitors with better pharmacological properties (such as those that do not cause drug-drug interactions with statins) may prove fruitful for the better treatment of cardiovascular disease and neurodegenerative diseases.

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