1. Introduction

Cardiovascular disease is one of the leading causes of mortality and morbidity in industrialised nations. Hypercholesterolaemia is one of a number of risk factors identified that influences the development and progression of atherosclerosis. While drugs such as HMGCoA reductase inhibitors or statins have been shown to significantly reduce cholesterol levels and the risk for cardiovascular disease, there is still a pressing need to identify other compounds that might further reduce the risk. One such class of drugs, currently in preclinical and clinical studies, is acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors. Two isoforms of ACAT have been identified; ACAT 1 has a more ubiquitous distribution in steroidogenic tissue, pancreas, intestine and macrophages and ACAT 2 is predominantly expressed in hepatocytes and intestines. In human tissues, ACAT 2 expression is high in foetal hepatocytes but declines in adult hepatocytes. Its expression remains unchanged in foetal and adult human intestines. AGT enzymes participate in the assembly of chylomicrons and very low density lipoproteins (triglyceride rich lipoproteins) and also in the formation of cholesteryl ester storage droplets within cells residing in the vessel wall. The initial results from preclinical and clinical studies suggest that ACAT inhibitors may have a beneficial effect in altering lipid profiles and in retarding the progression of atherosclerotic disease.

Keywords: acyl-CoA:cholesterol acyltransferase (ACAT), atherosclerosis, foam cells, hypercholesterolaemia, hyperlipidaemia, hypertriglyceridaemia

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Cardiovascular disease is one of the leading causes of mortality and morbidity in industrialised nations, Hypercholesterolaemia is one of a number of risk factors identified that influences the development and progression of atherosclerosis. While drugs such as HMGCoA reductase inhibitors or statins have been shown to significantly reduce cholesterol levels and the risk for cardiovascular disease, there is still a pressing need to identify other compounds that might further reduce the risk. One such class of drugs, currently in preclinical and clinical studies, is acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors. Two isoforms of ACAT have been identified; ACAT 1 has a more ubiquitous distribution in steroidogenic tissue, pancreas, intestine and macrophages and ACAT 2 is predominantly expressed in hepatocytes and intestines. In human tissues, ACAT 2 expression is high in foetal hepatocytes but declines in adult hepatocytes. Its expression remains unchanged in foetal and adult human intestines. AGT enzymes participate in the assembly of chylomicrons and very low density lipoproteins (triglyceride rich lipoproteins) and also in the formation of cholesteryl ester storage droplets within cells residing in the vessel wall. The initial results from preclinical and clinical studies suggest that ACAT inhibitors may have a beneficial effect in altering lipid profiles and in retarding the progression of atherosclerotic disease.


1. Introduction

With cardiovascular disease still a leading cause of death in industrialised nations, there is a continued effort to identify different treatment modalities. One of the earliest manifestations of atherosclerosis is the presence of cholesteryl ester (CE)-enriched macrophages and smooth muscle cells (foam cells) within the vessel wall [1]. One of the major mechanisms for foam cell formation is receptor and non-receptor mediated uptake of modified lipoproteins, such as low density lipoproteins (LDL) and very low density lipoproteins (VLDL) [2]. Internalised, modified lipoproteins are degraded in lysosomes and the hydrolysed, unesterified or free cholesterol (FC) can then be transported to the plasma membrane. Excess FC is re-esterified by the enzyme, acyl-CoA:cholesterol acyltransferase (ACAT), to form esterified cholesterol (EC) [3] (Scheme 1), a critical step in foam cell formation. It is this excess accumulation of EC droplets that imparts a foamy appearance to the cells under light microscopy.

Two isoforms of ACAT have been described, with type 1 having more ubiquitous distribution (i.e., liver, pancreas, macrophage, steroidogenic tissue) and type 2 being found exclusively in the liver and intestine [4]. ACAT plays an important role in the assembly of chylomicrons in the intestine (derived from dietary chole-
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Figure 1. Re-esterification of cholesterol.

endoplasmic reticulum

Unesterified cholesterol

ACAT

Esterified cholesterol

Fatty acyl-CoA

The pivotal role of ACAT in atherosclerosis and in lipoprotein metabolism has been determined through the use of ACAT inhibitors. Novel effects of the ACAT inhibitor, 58-035, during foam cell development in cultured primary human macrophages (HMM) have been recently reported. In the presence of a modified LDL (acylated LDL, acLDL), 58-035 significantly lowered cholesterol accumulation by two methods: by decreasing high affinity binding of 125I-acLDL and enhancing cholesterol efflux. The results showed that CI-1011 significantly decreased the cross-sectional lesion area and the monocyte-macrophage content of the foam cell rich aortic arch by 35 and 27%, respectively. In addition, CI-1011 decreased thoracic aorta and iliac-femoral CE concentrations by 39%, suggesting that CI-1011 had a direct inhibitory effect on ACAT activity within the vessel wall. The

Many ACAT inhibitors have been developed to reduce the formation and progression of atherosclerotic lesions in various in vivo animal models. One compound currently being studied in clinical trials is CI-1011 (avasimibe), an oxysulfonylcarbamate that inhibits ACAT function and is bioavailable. Deising et al. studied the effect of this inhibitor on atherosclerotic development and found that lesions were significantly lower in apolipoprotein E (apoE)-Leiden transgenic animals fed a cholesterol diet plus CI-1011, compared with control animals. In a study examining atherosclerotic regression, Bocan et al. reported that New Zealand white rabbits fed a high cholesterol, high fat diet and then treated with CI-1011 showed reduced atherosclerotic lesions compared with control animals. Cullen et al. reported greater FC efflux from HMM foam cells incubated with CI-1011 and/or Atorvastatin compared to control macrophages. There are currently clinical trials underway assessing the safety and efficacy of CI-1011 in patients with dyslipidemia and coronary artery disease. This compound appears to inhibit both ACAT isoforms and in preliminary clinical studies does have a favorable effect in altering lipid profiles. This review will discuss recent patent applications, from 2000 and selected published reports of preclinical and clinical studies with various non-specific ACAT inhibitors. For more comprehensive reviews of ACAT and its isoforms (types 1 and 2), readers are referred to the following references.

2. ACAT inhibitors under experimental and preclinical investigation

2.1 Pfizer

Investigators from Warner-Lambert (now Pfizer Global R&D) demonstrated that the combination of matrix metalloproteinase (MMP) inhibitors and ACAT inhibitors could reduce atherosclerotic burdens in animals. One of the ACAT inhibitors described, [2,4,6-tris(1-methylethyl)phenyl]oxysulfonyl-2,6-bis(1-methylethyl)phenylacetyl]sulfamic acid, 2,6-bis(1-methylethyl)phenylacetyl]sulfamic acid, (CI-1011), 1, is a bioavailable oxysulfonylcarbamate. The compound has been studied in a number of atherosclerotic animal models, including rats, rabbits, mini-pigs, hamsters and cynomolgous monkeys. A recent study examined the effect of CI-1011 on MMP expression in New Zealand white rabbits. The male rabbits were fed a cholesterol/fat diet for 9 weeks, fat only diet for 6 weeks and then CI-1011 (25 mg/kg) for 7 - 8 weeks. The results showed that CI-1011 significantly decreased the cross-sectional lesion area and the monocyte-macrophage content of the foam cell rich aortic arch by 35 and 27%, respectively. In addition, CI-1011 decreased the monocyte-macrophage content in the iliac-femoral artery by 77%. Without affecting plasma cholesterol levels, CI-1011 decreased thoracic aorta and iliac-femoral CE concentrations by 39%, suggesting that CI-1011 had a direct inhibitory effect on ACAT activity within the vessel wall. The
concentrations of CI-1011 in the plasma and in the tissues were 178 ng/ml and 25 ng/g of tissue wet weight, levels that were higher than necessary to inhibit macrophage ACAT (IC_{50} of 24 nmol/l or 12 ng/ml). The levels of MMP within the aortic arch were reduced by CI-1011 treatment. mRNA levels of MMP-9 and tissue inhibitor of MMP-1 and -2 (TIMP-1 and TIMP-2) also decreased to between 28 - 39%. These findings were associated with a reduction of macrophage area within the aortic arch and were attributed to decreases in macrophage number, not size. Thus, CI-1011 attenuated the development of atherosclerotic lesions by specifically altering the cellular composition of the lesions.

In a study by Nicolosi et al. [24], F1B male hamsters were fed a hypercholesterolaemic chow diet containing 10% coconut oil and 0.05% cholesterol, with or without varying concentrations of CI-1011 (3 - 30 mg/kg per day) or cholestyramine (500 mg/kg per day), for 10 weeks. The results showed that all treatments significantly lowered the aortic fatty streak area. Plasma cholesterol levels decreased by 25%, 32%, 34% and 32% in the animals treated with CI-1011 3, 10, 30 mg/kg per day and with cholestyramine, respectively. Similarly, plasma triglyceride levels were decreased by 48%, 47%, 42% and 45% respectively, with LDL cholesterol decreasing by 25%, 38%, 47% and 46%, respectively. HDL cholesterol levels were similar in all treatment groups. These results concluded that CI-1011 was significantly more potent than cholestyramine in lowering cholesterol and in reducing the aortic fatty streak area [24].

The effect of CI-1011 on apolipoprotein B-100 levels was also studied in human hepatoma cells (HepG2 cells) that were incubated with CI-1011 0.001 - 10 nmol/l for 24 h [25]. The results showed that apoB secretion decreased by 25 - 43% (p < 0.002). At the lowest doses CI-1011 inhibited HepG2 cellular ACAT activity by 79% (p < 0.002), lowered CE mass by 32% (p < 0.05), decreased apoB secretion by 42% (p = 0.019) and increased intracellular apoB degradation by 42% (p = 0.019).

2.2 Sankyo KK
Novel phenylenediamine derivatives, N-(2-butoxy-4-morpholinophenyl)-N'-(2,2-dimethyl-1-(pyridin-3-yl)propyl)urea, 2, claimed by Sankyo, have been shown to inhibit ACAT in rat liver microsomes [102]. The IC_{50} value for the specified compound was 0.12 µM. These compounds are also thought to inhibit lipid peroxidation.

Arylurea and arylmethylcarbamoyl derivatives, 3, claimed by Sankyo, have also been shown to inhibit ACAT in rat liver microsomes [103]. The specified compound, (S)-(-)-N-[4-(2-methylphenyl)-6-phenylpyridin-3-yl]-N-(2,2-dimethyl-1-(pyridin-3-yl)propyl)urea, potently inhibited ACAT with an IC_{50} value of 54.2 ng/ml.

2.3 Sumitomo Pharmaceutical
Novel naphthyridine derivatives developed by Sumitomo Pharmaceutical have been shown to inhibit ACAT in rat peritoneal macrophages [104]. The specified compound, N-[1-butyl-4-(3-methoxyphenyl)-1,2-dihydro-2-oxo-1,8-naphthyridin-3-yl]-N-[2-t-butyl-5-(morpholinomethyl)phenyl]urea, 4, potently inhibited ACAT by 97% at a concentration of 1 µM.

2.4 The Korean Research Institute of Bioscience and Biotechnology
Investigators from the Korean Research Institute of Bioscience and Biotechnology have shown that the bioflavonoid, neohesperidin dihydrochalcone, C_{28}H_{36}O_{15}, (MW 612.60), 5, is an ACAT inhibitor [105]. Neohesperidin dihydrochalcone is extracted from grapefruit and other citrus fruits and can also be synthesised from naringin. White Sprague Dawley rats were fed either control chow or chow supplemented with neohesperidin (0.05%) for 6 weeks. The results showed that plasma cholesterol levels decreased by 15% in neohesperidin fed animals, compared with controls. ACAT activity was assessed in liver microsomes isolated from these animals and the results showed that its activity was 20% lower in neohesperidin treated animals.
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In a study by Shin et al., 20 male Sprague Dawley rats with were fed either the control high cholesterol diet (1% wt/wt) or the same diet supplemented with naringin (0.1%, wt/wt) for 6 weeks [26]. The results showed that plasma and hepatic cholesterol decreased significantly, by 31% and 23%, respectively, while no significant changes were observed for plasma or hepatic triglyceride levels. HMGCoA reductase activity decreased by 24% and ACAT activity decreased by 20% in naringin fed animals compared with controls. It was also found that faecal excretion of neutral sterols was 2.5-fold higher in the control animals as compared with naringin treated animals. Similar results were found in rats fed narin- genin [27] and in rats fed either tangerine-peel extract or a mixture of naringen and hesperidin [28].

2.5 Pola Kasei Kogyo
Novel triterpenoids, ilekudinols A, B and C, from Pola Kasei Kogyo, were found to be ACAT inhibitors. The compounds were isolated from diethyl ether extracts of Ilex kudincha leaves [29,30]. Compound A, \(\text{6} \alpha,3\beta\)-dihydroxy-24-nor-urs-4(23),11-dien-28,13\(\beta\)-olide, inhibited ACAT activity in rat liver microsomes with an IC\(_{50}\) value of 70 \(\mu\)g/ml [106]. The two other triterpenoids described, compounds B and C, inhibited ACAT with IC\(_{50}\) values of 20 and 150 \(\mu\)g/ml, respectively.

Investigators have isolated novel polycyclic compounds from Ilex kudincha and have shown them to be ACAT inhibitors [107]. The specified compound, \(\text{7} \), was the most potent inhibitor with an IC\(_{50}\) value of 43.9 \(\mu\)M.

Other novel triterpenes from Pola Kasei Kogyo have also been found to be ACAT inhibitors. Four compounds are claimed and the specified compound, \(\text{8} \), (0.2 mg/ml) inhibited ACAT activity by 65.8% [108].

2.6 Sumimoto
Novel cycloalkyl derivatives from Sumimoto, have been shown to inhibit ACAT activity in rabbit liver microsomes. Compound \(\text{9} \), N-(2,2-diphenylcyclopropyl)-N'-[2,6-diisopropylphenyl]urea, inhibited ACAT with an IC\(_{50}\) value of 17 nM. Novel quinazolinone derivatives have been shown to be ACAT inhibitors [110]. The hydrochloride salt of the specified compound, N-(2,6-diisopropylphenyl)-[1-butyl-4-[3-(pyridin-3-yl)acetamide], \(\text{10} \), inhibited rat peritoneal macrophage microsomal ACAT activity with an IC\(_{50}\) value of 3.5 nM.

2.7 Mitsubishi Kagaku
Novel diphenylurea derivatives claimed by Mitsubishi Kagaku [111] were found to inhibit macrophage ACAT activity, with the specified compound, \(\text{11} \), having an IC\(_{50}\) value of 3.3 nM. A total of 31 compounds are described.

2.8 Other ACAT investigations
The above studies have utilised a variety of agents to inhibit ACAT in different animal models. In the aggregate, the results showed that ACAT inhibition was beneficial in lowering cholesterol levels and decreasing atherosclerotic burden, without causing serious hematologic or biochemical damage to the animals studied.

The results from ACAT1 knockout mice, with or without LDL receptor or apo-E deficiency, have yielded opposite results. Fazio et al., developed a transplantation model that utilised LDL-receptor deficient mice transplanted with marrow or foetal liver cells that lacked ACAT1 [31]. While there was no statistically significant change in serum lipid chemistries, they did find that atherosclerotic lesion size increased significantly in LDL\(^{-/-}\) mice that were recipients of ACAT1\(^{-/-}\) marrow. They noted that there was a death of macrophages in these lesions. Using TUNEL staining of aortic cross sections, the investigators showed that cells were 3-fold higher in ACAT1\(^{-/-}\) recipient mice compared with controls. This suggested that apoptosis contributed to reduced macrophage number within the lesions [31].
The ApoE-/- mice were fed a western diet for 17 weeks with on atherosclerosis and cytotoxicity in apoE deficient mice. The investigators examined the effect of partial ACAT inhibition in an animal that was severely hyperlipidaemic. In a recent report by Kusunoki et al. [33], these investigators found that rutin and quercetin (bioflavonoids found abundantly in herbs, vegetables, grains and fruits) inhibited rat liver microsomal ACAT activity by 25% and 20%, respectively, as compared to the control mice [33]. These results suggested that partial ACAT deficiency had anti-atherogenic effects.

3. ACAT Inhibitors under clinical investigation

Investigators from Pfizer, have examined the efficacy and short-term safety of the ACAT inhibitor, CI-1011, on the lipid profile in patients with mixed hyperlipidaemia [112]. Patients with hypertriglyceridaemia, hypercholesterolaemia and low HDL cholesterol levels were treated with either placebo or varying doses of CI-1011 (50, 125, 250, or 500 mg/day) for 8 weeks. The goal of the study was to evaluate the effect of CI-1011 on lipid parameters, cortisol and insulin levels and the side effect profile. During the course of the study, the investigators found that CI-1011 did not alter cortisol or insulin levels and the side effect profiles did not differ between the placebo treated group and patients taking the active compound. They did find that all doses of CI-1011 significantly reduced levels of VLDL cholesterol and total triglyceride, without altering levels of total cholesterol, LDL, HDL, apoA-1 or apoB. Cortisol and insulin levels were similarly unaffected and the drug did not produce any biochemical, haematologic, or clinical abnormalities in the study patients. It was concluded that CI-1011 was safe and had favourable effects in reducing triglyceride levels. Other clinical studies are currently underway with CI-1011, including studies examining endothelial function, myocardial activity by positron emission tomography and the safety and efficacy of combination therapy with statin.

3.1. The Korea Research Institute for Bioscience and Biotechnology

Investigators have examined the effects of rutin (C13H42O16, MW 630.51 Da), [12] and quercetin (C15H10O7, MW 302.23 Da), [13] on the lipid profile, HMGCoA reductase activity and ACAT activity in rats [113]. The investigators found that rutin and quercetin (bioflavonoids found abundantly in herbs, vegetables, grains and fruits) inhibited rat liver microsomal ACAT activity by 25% and 20%, respectively, as compared with the control animals. They also studied the effect of rutin in two men in their mid-50s. Study subjects were given a daily oral dose of rutin (10 mg/kg) for 60 days, which decreased plasma cholesterol and neutral lipid contents by 25% and 20%, respectively.

The effect of cinnamic acid, [14] on the lipid profile and ACAT activity in rats was claimed by the Korea Research Institute for Bioscience and Biotechnology [114]. Cinnamic acid is a precursor in bioflavonoid synthesis and can be extracted from a number of fruits and plants, including apple, grape, strawberry, plum, cherry, blueberry, potato, tea, coffee or nuts. It was reported that cinnamic acid and derivatives inhibited rat liver microsomal ACAT activity by 7-20%. Two men in their mid-50s were administered a daily oral dose of 4-hydroxycinnamic acid for 60 days. As compared with baseline levels, plasma cholesterol and triglyceride levels decreased 25% and 20%, respectively.

3.2 Other investigations on ACAT inhibitors

There have only been a few other studies examining the role of different ACAT inhibitors on cholesterol metabolism in humans, with many focused on decreasing intestinal cholesterol absorption. To the best of our knowledge, none other than CI-1011 is currently being studied in early phase...
clinical trials. However, an earlier study in 1980 examined the effect of the ACAT inhibitor, CL 277,082 on cholesterol metabolism in eight healthy male donors [34]. Subjects were given placebo for 14 days and then an oral dose of CL 277,082 (750 mg/day) for 20 days. The medication was well-tolerated but cholesterol absorption, sterol excretion rates and plasma lipoprotein levels were not significantly different between the two treatment groups [34]. It was concluded that the single dose of CL 277,082 might not have been therapeutic.

In 1994, Hainer et al. [35] examined the effect of a poorly absorbed ACAT inhibitor, DUP128, on cholesterol absorption in 14 different study subjects. Study subjects were given varying doses of DUP128 (900, 1800 or 3600 mg/day), neomycin (1 gm b.i.d.) or placebo for 7 weeks. The results showed that DUP128 significantly reduced cholesterol absorption compared with placebo and significantly reduced levels of LDL. The conclusion was that DUP128 reduced cholesterol absorption but the effects were small.

In 1995, Peck et al. [36] examined the tolerability and pharmacokinetics of the ACAT inhibitor, 447C88, on a group of healthy male volunteers. Study participants were double-blinded and received placebo or single doses of 447C88 (25, 50, 100, 200, 400 and 800 mg) with food. Giving subjects the 400 mg dose, after an overnight fast, tested bioavailability of the compound. Different study subjects received placebo or a single 200 mg dose of 447C88. The results showed no significant changes in the lipid profile. The plasma concentration of 447C88 was not detectable after the overnight fast and was low after the other doses.

4. Expert opinion

The role of ACAT in cholesterol absorption, lipoprotein assembly and foam cell formation has made it an obvious target for intervention in the treatment of dyslipidaemia and atherosclerosis. A number of different structural forms of non-specific ACAT inhibitors have been evaluated in animals and in humans. It appears from the work of Chang et al. [9] that targeting ACAT1 might not be very promising, as it appears to decline in expression in adult human hepatocytes. The experimental and preclinical studies in various animal models have shown favourable reductions in cholesterol and triglyceride levels, along with reductions in atherosclerotic burden. Genetically engineered mice deficient in ACAT1 (either alone or bred as a double knockout deficient in LDL receptors or apolipoprotein E) have also shown reductions in plasma cholesterol levels when fed normal or high fat Chow diets. The discrepancy between animals fed cholesterol inhibitors (mostly rabbit modeling) and the double ACAT1 knockout mice resides in the atherosclerotic burden and the presence of extracellular FC accumulation. The double ACAT1 knockout mice (particularly LDL1-/-transplanted with bone marrow macrophages derived from the ACAT1, knockout) appeared to have increased FC accumulation within the vessel wall and more extensive aortic lesions compared with control mice [10]. The obvious difference between the two experimental models resides in the degree of ACAT deficiencies. It would appear that the complete absence of ACAT1 in the background of severe hyperlipidaemia would pose an increased risk for cholesterol xanthomatosis and atherosclerotic disease. Such complete deficiencies of ACAT1 and severe hyperlipidaemia have not been described in humans. The results in [16] mice fed F-1394 suggest that partial ACAT deficiency has antiatherogenic effects [19]. Those patients currently taking the ACAT inhibitor, CI-1011, appear to tolerate the medication without significant adverse effects [19]. In vitro studies of cultured primary human macrophages, simultaneously exposed to the ACAT inhibitors, F-1394 and acyl-CoA acyltransferase inhibitors on a group on healthy male volunteers. Study participants were double-blinded and received placebo or single doses of 447C88 (25, 50, 100, 200, 400 and 800 mg) with food. Giving subjects the 400 mg dose, after an overnight fast, tested bioavailability of the compound. Different study subjects received placebo or a single 200 mg dose of 447C88. The results showed no significant changes in the lipid profile. The plasma concentration of 447C88 was not detectable after the overnight fast and was low after the other doses.

Bibliography


** This article focuses on expression of ACAT enzymes in human tissues, and concludes...
that there appear to be species differences, particularly with ACAT inhibitors. These findings suggest that targeting ACAT, for inhibition in humans might not be helpful.


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Patents
Papers of special note have been highlighted as either of interest (+) or of considerable interest (**+) to readers.

** The only ACAT inhibitor currently being tested in clinical trials.