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Update on the role of acyl-CoA:cholesterol acyltransferase inhibitors in atherosclerosis

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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. ACAT 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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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) [2,3]. 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) [2] (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, macrophages, steroidogenic tissue) and type 2 being found exclusively in the liver and intestine [4,5]. ACAT plays an important role in the assembly of chylomicrons in the intestine (derived from dietary choles-

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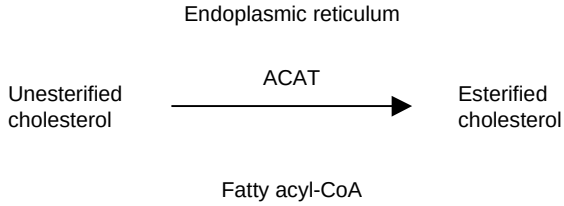


Figure 1. Re-esterification of cholesterol.

terol and fat) and VLDL in the liver [5,6]. Within the intestine, ACAT serves to esterify dietary FC to facilitate its transport to the liver for processing. In the liver, ACAT presumably serves as a synthetic enzyme for the formation of triacylglycerols, constitutive components of lipoproteins that are secreted from the liver to peripheral tissues. Within cells of the vessel wall, $ACAT_1$ expression has been shown to be upregulated during monocyte to macrophage differentiation and foam cell formation [7] and high levels have also been found in atherosclerotic lesions [8].

$ACAT_2$ appears to have a variable level of expression in human hepatocytes and in the intestinal epithelium. In human tissues, $ACAT_2$ appears to be highly expressed in foetal hepatocytes, but then essentially disappears in adult hepatocytes [9]. $ACAT_2$ expression does not appear to diminish in human adult intestines as compared with human foetal intestines. As compared to $ACAT_1$, human $ACAT_2$ is localised to the villus, while $ACAT_1$ has a more uniform distribution along the villus-crypt [9]. There may be species differences in $ACAT_2$ expression as in non-human primates, $ACAT_2$ is expressed in adult monkey hepatocytes, whereas $ACAT_1$ appears to be confined to cells lining interhepatocellular spaces [10].

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 [11]. In the presence of a modified LDL (acetylated LDL, acLDL), 58-035 significantly lowered cholesterol accumulation by two methods: by decreasing high affinity binding of ^{125}I-acLDL and enhancing cholesterol efflux [11]. Additionally, 58-035 was not cytotoxic to the cells [11]. Further studies using another ACAT inhibitor, CI-1011, in cultured human macrophages, demonstrated that the inhibitor reduced B_{max} for ^{125}I-acLDL, suggesting that the compound directly inhibited expression of scavenger receptors [12]. The pattern of decreased lipid accumulation in HMMs exposed to acLDL plus 58-035 was consistent with those reported by Meiner *et al.* [13]; showing that total cholesterol and EC accumulation in peritoneal macrophages isolated from ACAT knockout mice and exposed to acLDL were lower than in control macrophages.

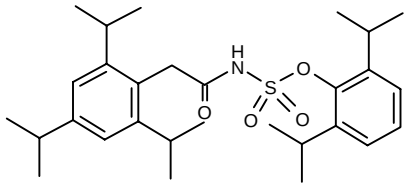
Many ACAT inhibitors have been developed to reduce the formation and progression of atherosclerotic lesions in various *in vivo* animal models [14]. One compound currently being studied in clinical trials is CI-1011 (avasimibe), an oxysulfonylcarbamate that inhibits ACAT function and is bioavailable [15]. Delsing *et al.* studied the effect of this inhibitor on atherosclerosis 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 [16]. 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 [17]. Cullen *et al.* reported greater FC efflux from HMM foam cells incubated with CI-1011 and/or Atorvastatin compared to control macrophages [18]. There are currently clinical trials underway assessing the safety and efficacy of CI-1011 in patients with dyslipidaemia and coronary artery disease. This compound appears to inhibit both ACAT isoforms [15] and in preliminary clinical studies does have a favourable effect in altering lipid profiles [19].

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 [20,21,22,23]. In addition, there is no clear distinction in the patent applications of whether compounds specifically inhibit $ACAT_1$ or $ACAT_2$.

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]acetyl]sulfamic acid, 2,6-bis(1-methylethyl)phenyl ester, (CI-1011), 1, is a bioavailable oxysulfonylcarbamate [15,101]. The compound has been studied in a number of atherosclerotic animal models, including rats, rabbits, minipigs, hamsters and cynomologus monkeys [15]. A recent study examined the effect of CI-1011 on MMP expression in New Zealand white rabbits [17]. 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 [17]. 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



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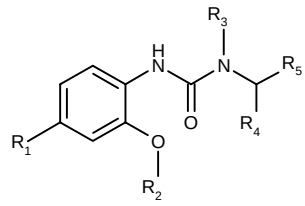

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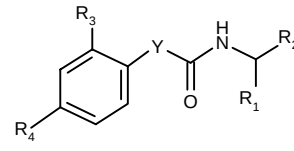

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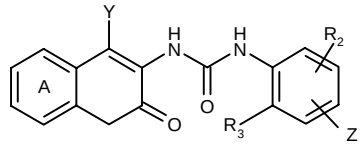

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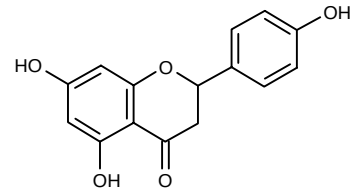

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concentrations of CI-1011 in the plasma and in the tissues were 178 ng/ml and 25 ng/g of tissue wet weight, levels that were higher than necessary to inhibit macrophage ACAT (IC_{50} of 24 nmol/l or 12 ng/ml). The levels of MMP within the aortic arch 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], F_1B 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% ($p < 0.0012$). At the lowest doses CI1011 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.

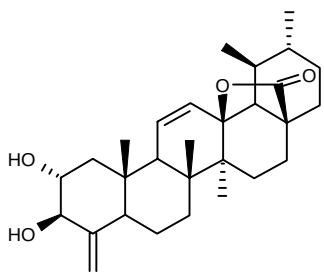
Arylurea and arylmethylcarbamoyl derivatives, 3, claimed by Sankyo, have also been shown to inhibit ACAT in rat liver microsomes [103]. The specified compound, (*S*)-(-)-*N*-[4-(2-methylphenyl)-6-phenylpyridin-3-yl]-*N*-[2,2-dimethyl-1-(pyridin-3-yl)propyl]urea, potently inhibited ACAT with an IC_{50}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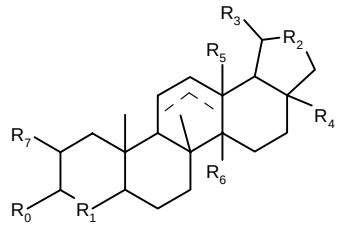

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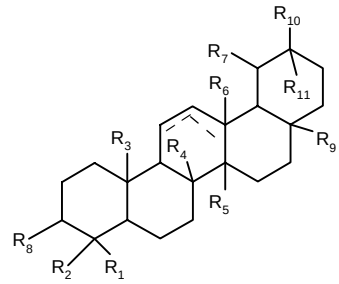

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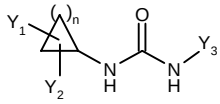

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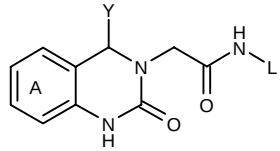

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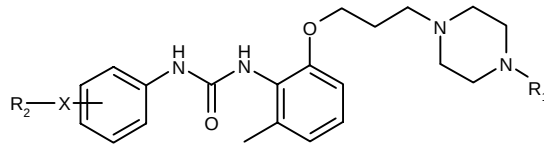

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In a study by Shin *et al.* 20 male Sprague Dawley rats with were fed either the control high cholesterol diet (1% wt/wt) or the same diet supplemented with naringin (0.1%, wt/wt) for 6 weeks [26]. The results showed that plasma and hepatic cholesterol 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 naringenin [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, 6, 2α,3β-dihydroxy-24-nor-urs-4(23),11-dien-28,13β-olide, inhibited ACAT activity in rat liver microsomes with an IC_{50} value of 70 μg/ml [106]. The two other triterpenoids described, compounds B and C, inhibited ACAT with IC_{50} values of 20 and 150 μg/ml, respectively.

Investigators have isolated novel polycyclic compounds from *Ilex kudincha* and have shown them to be ACAT inhibitors [107]. The specified compound, 7, was the most potent inhibitor with an IC_{50} value of 43.9 μM.

Other novel triterpenes from Pola Kasei Kogyo have also been found to be ACAT inhibitors. Four compounds are claimed and the specified compound, 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 [109]. Compound 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-ylmethoxy)phenyl]-3,4-dihydro-2-oxo-1H-quinazolin-3-yl]acetamide, 10, inhibited rat peritoneal macrophage microsomal ACAT activity with an IC_{50} value of 3.5 nM.

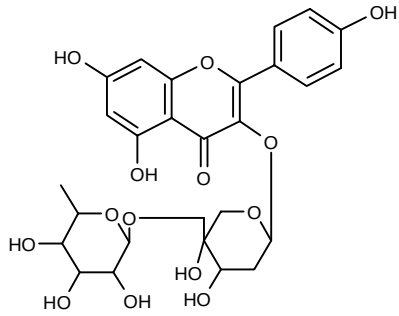
2.7 Mitsubishi Kagaku

Novel diphenylurea derivatives claimed by Mitsubishi Kagaku [111] were found to inhibit macrophage ACAT activity, with the specified compound, 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 haematologic or biochemical damage to the animals studied.

The results from $ACAT_1$ 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 $ACAT_1$ [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 $ACAT_1^{-/-}$ 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 $ACAT_1^{-/-}$ recipient mice compared with controls. This suggested that apoptosis contributed to reduced macrophage number within the lesions [31].



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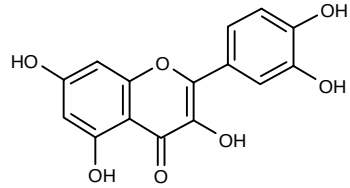

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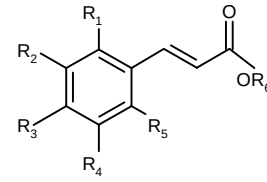

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In addition, there have been reports that have shown excessive blockade of the ACAT enzyme can lead to cutaneous lesions and cerebral cholesterol calcifications. Histological examination of similar lesions in the same subset of transgenic mice, performed by Accad *et al.*, showed a decrease in the number of macrophage-type foam cells in fatty streak lesions sectioned from the aorta; they also tended to have decreased amounts of neutral lipids [32]. This group had similar findings with regards to the development of cutaneous xanthomatosis, as well as significant crystalline deposits in the brain. However, none of these results have been observed in investigational trials on human subjects at present.

The increased cytotoxicity observed in the studies of Fazio *et al.* [31] and Accad *et al.* [32] might have been due to the complete absence of $ACAT_1$ in an animal that was severely hyperlipidaemic. In a recent report by Kusunoki *et al.* [33], these investigators examined the effect of partial ACAT inhibition on atherosclerosis and cytotoxicity in apoE deficient mice. The $ApoE^{-/-}$ mice were fed a western diet for 17 weeks with and without the ACAT inhibitor F-1394 (inhibits both isoforms, at either 300 mg/kg or 900 mg/kg dose). The results showed significantly less atherosclerotic burden and no evidence for cytotoxicity in mice fed either dose of F-1394, 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 [19,101]. 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 atorvastatin.

3.1 The Korea Research Institute for Bioscience and Biotechnology

Investigators have examined the effects of rutin ($C_{27}H_{30}O_{16}$, MW 610.51 Da), 12 [112] and quercetin ($C_{15}H_{10}O_7$, 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 focussed 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 1990, 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 [35]. 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 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. 14 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 in 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 $ACAT_2$ 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 $ACAT_1$ (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 models) and the double $ACAT_1$ knockout mice resides in the atherosclerotic burden and the presence of extracellular FC accumulation. The double $ACAT_1$ knockout mice (particularly $LDL^{-/-}$ transplanted with bone marrow macrophages derived from the $ACAT_1$ knockout) appeared to have increased FC accumulation within the vessel wall and more extensive aortic lesions compared with control mice [31]. The obvious difference between the two experimental models resides in the degree of ACAT deficiency. It would appear that the complete absence of $ACAT_1$ in the background of severe hyperlipidaemia would pose an increased risk for cholesterol xanthomatosis and atherosclerotic disease. Such complete deficiencies of $ACAT_1$ and severe hyperlipidaemia have not been described in humans. The results in $apoE^{-/-}$ mice fed F-1394 suggest that partial ACAT deficiency has anti-atherogenic effects [33]. 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, 58-035 and CI-976 and acetylated LDL did not show evidence for cytotoxicity and FC accumulation was not significantly increased [11]. The next step is to await the results from the ongoing trials with CI-1011 to determine whether ACAT inhibition in the treatment of patients with cardiovascular disease provides promising results.

Bibliography

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

1. ROSS R: The pathogenesis of atherosclerosis: an update. *N. Engl. J. Med.* (1986) 314:488.
2. BROWN MS, HO HL, GOLDSTEIN JL: The cholesteryl ester cycle in macrophage foam cells. *J. Biol. Chem.* (1980) 255:9344-9352.
3. GUYTON JR, KLEMP KF: Development of the lipid-rich core in human atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* (1996) 16:4-11.
4. CASES S, NOVAK S, ZHENG Y-W: ACAT-2, a second mammalian acyl-CoA:cholesterol acyltransferase. *J. Biol. Chem.* (1998) 273:26755-26764.
5. BUHMAN KF, ACCAD M, FARESE RV JR.: Mammalian acyl-CoA:cholesterol acyltransferases. *Biochim. Biophys. Acta.* (2000) 1529:142-154.

•• This review article highlights recent studies of the molecular expression of $ACAT_1$ and $ACAT_2$.

6. CHANG TY, CHANG CCY, CHENG D: Acyl-CoenzymeA:cholesterol acyltransferase. *Ann. Rev. Biochem.* (1997) 66:613-638.
7. WANG H, GERMAIN SJ, BENFIELD PB *et al.*: Gene expression of acyl coenzyme A:cholesterol acyltransferase is upregulated in human monocytes during differentiation and foam cell formation. *Arterioscler. Thromb. Vasc. Biol.* (1996) 16:809-814.
8. MIYAZAKI A, SAKASHITA N, LEE O *et al.*: Expression of ACAT-1 protein in human atherosclerotic lesions and cultured human monocytes-macrophages. *Arterioscler. Thromb. Vasc. Biol.* (1998) 18:1568-1574.
9. CHANG CYC, SAKASHITA N, ORNVOLD K *et al.*: Immunological quantitation and localization of ACAT-1 and ACAT-2 in human liver and small intestine. *J. Biol. Chem.* (2000) 275:28083-28092.

•• This article focuses on expression of ACAT enzymes in human tissues, and concludes

that there appear to be species differences, particularly with $ACAT_2$. These findings suggest that targeting $ACAT_2$ for inhibition in humans might not be helpful.

10. LEE RG, WILLINGHAM MC, DAVIS MA *et al.*: Differential expression of $ACAT_1$ and $ACAT_2$ among cells within liver, intestine, kidney, and adrenal of nonhuman primates. *J. Lipid Res.* (2000) 41:1991-2001.

11. RODRIGUEZ A, BACHORIK PS, WEE S-B: Novel effects of the acyl-Coenzyme A:cholesterol acyltransferase inhibitor 58-035 on foam cell development in primary human monocyte-derived macrophages. *Arterioscler. Thromb. Vasc. Biol.* (1999) 19:2199-2206.

• This article examines novel effects of the non-specific ACAT inhibitor, 58-035, on foam cell formation in human macrophages. The results suggested that the inhibitor did not induce cytotoxicity, a finding that contrasted with increased cytotoxicity of ACAT inhibition in cultured murine macrophages.

12. RODRIGUEZ A, USHER D: Anti-atherogenic effects of the acyl-CoA:cholesterol acyltransferase, Avasimibe (CI-1011), in cultured primary human macrophages. *Atherosclerosis* (2001) In Press.

13. MEINER VL, CASES S, MYERS HM *et al.*: Disruption of the acyl-CoA:cholesterol acyltransferase gene in mice: Evidence suggesting multiple cholesterol esterification enzymes in mammals. *Proc. Natl. Acad. Sci. USA* (1996) 93:14041-14046.

14. MATSUDA K: ACAT inhibitors as antiatherosclerotic agents: compounds and mechanisms. *Med. Res. Rev.* (1994) 14:271-305.

• A comprehensive review of the structurally different compounds that are ACAT inhibitors.

15. SORBERA LA, RABASSEDA X, CASTANER J: Avasimibe, treatment of lipoprotein disorders. *Drugs Fut.* (1999) 24:9-15.

16. DELSING DJM, OFFERMAN EH, VAN DER ROOM H *et al.*: The acyl-CoA:cholesterol acyltransferase (ACAT)-inhibitor avasimibe reduces atherosclerosis independently of its lipid-lowering effect in cholesterol-fed apoE3-Leiden transgenic mice. *Circulation* (Suppl.) (1999) Abstract 3232.

17. BOCAN TMA, KRAUSE BR, ROSEBURY WS *et al.*: The ACAT inhibitor avasimibe reduces macrophages and matrix metalloproteinase expression in atherosclerotic lesions of hypercholesterolemic rabbits. *Arterioscler. Thromb. Vasc. Biol.* (2000) 20:70-79.

18. CULLEN P, CIGNARELLA A, ASSMANN G: The effects of atorvastatin, the ACAT inhibitor CI-1011, and progesterone on storage and secretion of cholesterol in human monocyte-derived macrophages. *Circulation* (1998) Abstract 2828.

19. INSULL W, KOREN M, DAVIGNON J *et al.*: Efficacy and short-term safety of a new ACAT inhibitor, avasimibe, on lipids, lipoproteins, and apolipoproteins, in patients with combined hyperlipidemia. *Atherosclerosis* (2001) In Press.

• A recent publication examining the effects of CI-1011 in patients. The results showed a significant decrease in triglyceride levels, but no apparent increase in side effects.

20. WU J, DOSHI R, RODRIGUEZ A: An update of acyl-CoA:cholesterol acyltransferase inhibition in animal and human *in vitro* and *in vivo* models of atherosclerosis. *Curr. Med. Chem.-Imm. Endoc. & Metab. Agents* (2001) 1:141-150.

21. RUDEL LL, LEE RG, COCKMAN TL: Acyl coenzyme A: cholesterol acyltransferase types 1 and 2: structure and function in atherosclerosis. *Curr. Opin. Lipidol.* (2001) 12:121-127.

22. BUHMAN KF, ACCAD M, FARESE RV JR: Mammalian acyl-CoA:cholesterol acyltransferases. *Biochim. Biophys. Acta.* (2000) 1529:142-154.

23. JOYCE C, SKINNER K, ANDERSON RA *et al.*: Acyl-coenzyme A:cholesterol acyltransferase 2. *Curr. Opin. Lipidol.* (1999) 10:89-95.

24. NICOLOSI RJ, WILSON TA, KRAUSE BR: The ACAT inhibitor, CI-1011 is effective in the prevention and regression of aortic fatty streak in hamsters. *Atherosclerosis* (1998) 137:77-85.

25. WILCOX LJ, BARRETT PHR, NEWTON RS *et al.*: ApoB100 secretion from HepG2 cells is decreased by the ACAT inhibitor CI-1011. *Arterioscler. Thromb. Vasc. Biol.* (1999) 19:939-949.

26. SHIN YW, BOK S-H, JEONG T-S *et al.*: Hypocholesterolemic effect of naringin associated with hepatic cholesterol regulating enzyme changes in rats. *Int. J. Vitam. Nutr. Res.* (1999) 69:341-347.

27. LEE SH, PARK YB, BAE KH *et al.*: Cholesterol-lowering activity of naringenin *via* inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme-A:cholesterol acyltransferase in rats. *Ann. Nutr. Metab.* (1999) 43:173-180.

28. BOK S-H, LEE S-H, PARK Y-B *et al.*: Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase and acyl CoA:cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. *J. Nutr.* (1999) 129:1182-1185.

29. NISHIMURA K, FUKUDA T, MIYASE T *et al.*: Activity-guided isolation of triterpenoid acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors from *Ilex kudincha*. *J. Nat. Prod.* (1999) 62:1061-1064.

30. NISHIMURA K, MIYASE T, NOGUCHI H: Triterpenoid saponins from *Ilex kudincha*. *J. Nat. Prod.* (1999) 62:1128-1133.

31. FAZIO S, MAJOR SS, SWIFT LL *et al.*: Increased atherosclerosis in LDL receptor-null mice lacking $ACAT_1$ in macrophages. *J. Clin. Invest.* (2001) 107:163-171.

32. ACCAD M, SMITH SJ, NEWLAND DL *et al.*: Massive xanthomatosis and altered composition of atherosclerotic lesions in hyperlipidemic mice lacking acyl CoA:cholesterol acyltransferase 1. *J. Clin. Invest.* (2000) 105:711-719.

33. KUSUNOKI J, HANSOTY DK, ARAGANE K *et al.*: Acyl-CoA:cholesterol acyltransferase inhibition reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation* (2001) 103:2604-2609.

34. HARRIS WS, DUJOVNE CA, VON BERGMANN K *et al.*: Effects of the ACAT inhibitor CL 277, 082 on cholesterol metabolism in humans. *Clin. Pharmcol. Ther.* (1990) 48:189-194.

35. HAINER JW, TERRY JG, CONNELL JM *et al.*: Effect of the acyl-CoA:cholesterol acyltransferase inhibitor DUP128 on cholesterol absorption and serum cholesterol in humans. *Clin. Pharmacol. Ther.* (1994) 56:65-74.

36. PECK RW, WIGGS R, POSNER J: The tolerability, pharmacokinetics and lack of effect on plasma cholesterol of 447C88, an acyl-CoA:cholesterol acyltransferase (ACAT) inhibitor with low bioavailability, in healthy volunteers. *Eur. J. Clin.*

Pharmacol. (1995) 49:243-249.

Patents

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

101. PFIZER GLOBAL R&D.: W00004892 (2000).

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

102. SANKYO KK: JP12026293 (2000).

103. SANKYO KK: JP12026294 (2000).

104. SUMITOMO PHARM. CO. LTD.: W00009505 (2000).

105. KOREA RESEARCH INSTITUTE OF BIOSCIENCE AND BIOTECHNOLOGY: W00015047 (2000).

106. POLA KASEI KOGYO KK: JP12044594 (2000).

107. POLA KASEI KOGYO KK: JP12044478 (2000).

108. POLA KASEI KOGYO KK: JP12256392 (2000).

109. SUMITOMO SEIYAKYU KK: JP12290246 (2000).

110. SUMITOMO SEIYAKU KK: JP12309576 (2000).

111. MITSUBISHI KAGAKU KK: JP12143629 (2000).

112. KOREA RESEARCH INST. OF BIOSCI. AND BIOTECH.: W00015237 (2000).

113. KOREA RESEARCH INST. OF BIOSCI. AND BIOTECH.: W00015237 (2000).

114. KOREA RESEARCH INST. OF BIOSCI. AND BIOTECH.: W00015215 (2000).

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