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The ACAT inhibitor avasimibe increases the fractional clearance rate of postprandial triglyceride-rich lipoproteins in miniature pigs

John R. Burnett [a], Dawn E. Telford [b], P. Hugh R. Barrett [c], Murray W. Huff [b,*]

[a] *Department of Core Clinical Pathology and Biochemistry, Pathwest Laboratory Medicine WA, Royal Perth Hospital and School of Medicine and Pharmacology, University of Western Australia, Perth, Australia*

[b] *Departments of Medicine and Biochemistry and Robarts Research Institute, 4-16, University of Western Ontario, 100 Perth Drive, London, Ontario, Canada N6A 5K8*

[c] *School of Medicine and Pharmacology, University of Western Australia, Perth, Australia*

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Abstract

Previously, we have shown, in vivo, that the acyl coenzyme A: cholesterol acyltransferase (ACAT) inhibitor avasimibe decreases hepatic apolipoprotein (apo) B secretion into plasma. To test the hypothesis that avasimibe modulates postprandial triglyceride-rich lipoprotein (TRL) metabolism in vivo, an oral fat load (2 g fat/kg) containing retinol was given to 9 control miniature pigs and to 9 animals after 28 days treatment with avasimibe (10 mg/kg/day, $n=5$; 25 mg/kg/day, $n=4$). The kinetic parameters for plasma retinyl palmitate (RP) metabolism were determined by multi-compartmental modeling using SAAM II. Avasimibe decreased the 2-h TRL ($d<1.006$ g/mL; $S_f>20$) triglyceride concentrations by 34%. The TRL triglyceride 0–12 h area under the curve (AUC) was decreased by 21%. In contrast, avasimibe had no effect on peak TRL RP concentrations, time to peak, or its rate of appearance into plasma, however, the TRL RP 0–12 h AUC was decreased by 17%. Analysis of the RP kinetic parameters revealed that the TRL fractional clearance rate (FCR) was increased 1.4-fold with avasimibe. The TRL RP FCR was negatively correlated with very low density lipoprotein (VLDL) apoB production rate measured in the fasting state ($r=-0.504$). No significant changes in total intestinal lipid concentrations were observed. Thus, although avasimibe had no effect on intestinal TRL secretion, plasma TRL clearance was significantly increased; an effect that may relate to a decreased competition with hepatic VLDL for removal processes.
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Keywords: ACAT inhibitor; Avasimibe; Triglyceride-rich lipoprotein; Tracer kinetic; Compartmental model

1. Introduction

Intracellular cholesterol esterification catalysed by the microsomal enzyme acyl coenzyme A: cholesterol acyltransferase (ACAT; EC 2.3.1.26) plays an important role in the development of atherosclerosis [1,2]. ACAT is present in a variety of tissues and the regulation of ACAT is necessary for cholesterol homeostasis. ACAT-derived cholesteryl esters are incorporated into both intestinal and hepatic lipoproteins, thus ACAT activity may modulate plasma cholesterol levels. Furthermore, ACAT derived cholesteryl esters accumulate within macrophages and smooth muscle cells in the arterial wall resulting in foam cell formation; a hallmark of early atherosclerosis. Therefore, an understanding of the regulation of ACAT should allow insights into the physiological functions of this enzyme that impact on the atherogenic process.

ACAT is an attractive therapeutic target in hypercholesterolemia and atherosclerosis [3] and ACAT inhibition may play an important role in its treatment [4]. Inhibitors of ACAT decrease plasma cholesterol concentrations in a number of small animal models [5]. However, most ACAT inhibitors show poor systemic bioavailability and thus, the primary mechanism of these compounds has been ascribed to the inhibition of

Abbreviations: ACAT, acyl coenzyme A: cholesterol acyltransferase; apo, apolipoprotein; AUC, area under the curve; ER, endoplasmic reticulum; FCR, fractional clearance rate; HDL, high density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HPLC, high performance liquid chromatography; HSPG, heparan sulfate proteoglycans; LDL, low density lipoprotein; LPL, lipoprotein lipase; LRP, LDL receptor related protein; RP, retinyl palmitate; S_f, Svedberg flotation; TRL, triglyceride-rich lipoprotein; VLDL, very low density lipoprotein

* Corresponding author. Tel.: +1 519 663 3793; fax: +1 519 663 3112.
E-mail address: mhuff@uwo.ca (M.W. Huff).

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cholesterol absorption [1,2]. Moreover, a lack of efficacy and/or toxicity after oral administration of ACAT inhibitors to humans or to animals fed diets containing physiologically relevant amounts of fat and cholesterol have limited their potential usefulness.

Recently, two different ACAT genes, *ACAT1* and *ACAT2* that code for the enzymes, ACAT1 and ACAT2 were identified and cloned [6–8]. ACAT1 is expressed in many tissues including macrophages and atherosclerotic lesions. ACAT2 (expressed in the liver and intestine) has recently been shown to be the primary isoform within human hepatocytes [9]. Although current ACAT inhibitors lack selectivity of ACAT1 versus ACAT2, specific inhibition of ACAT2, by reducing intestinal absorption of dietary cholesterol and decreasing secretion of hepatic lipoproteins, may lower plasma cholesterol concentrations and prevent atherosclerosis [10,11]. Studies in 'knockout' mouse models show decreased atherosclerosis when ACAT2 was deficient [12], however, ACAT1 deficiency did not prevent atherosclerotic lesion development [13]. Ideally, ACAT inhibitors should be absorbed and inhibit cholesterol esterification linked to both lipoprotein secretion as well as arterial wall macrophage foam cell formation [5].

Avasimibe (previously known as CI-1011) is an orally bioavailable inhibitor of ACAT [14–16]. This compound decreased plasma cholesterol [15,17] and triglyceride [18] concentrations, and reduced early atherosclerotic lesion development [19–23] in a variety of small animal models. In HepG2 cells, avasimibe decreases apolipoprotein (apo) B secretion; an effect associated with increased intracellular degradation [24,25]. Furthermore, in the same cell-line, it was shown that avasimibe does not affect triglyceride or phospholipid synthesis [24]. Avasimibe stimulates bile acid synthesis in primary rat hepatocytes by increasing the supply of free cholesterol both as substrate and by induction of cholesterol 7α-hydroxylase [26]. A significant decrease in plasma triglyceride concentrations was observed in combined hyperlipidemic human subjects with no effect on total cholesterol, low-density lipoprotein (LDL) cholesterol, or apoB levels [27]. Furthermore, avasimibe was not effective in monotherapy in subjects with homozygous familial hypercholesterolemia, but in combination modestly improved the total cholesterol-lowering effects of atorvastatin [28].

ApoB kinetic studies from our laboratory provided the first in vivo evidence, in a large animal model (miniature pigs fed a low-fat, cholesterol-free diet), that inhibition of ACAT (using intravenous DuP128) decreases hepatic apoB secretion [29]. A subsequent study showed that in pigs fed a diet higher in fat, intravenous DuP128 caused a more modest reduction in VLDL apoB secretion [30]. Hepatic microsomal ACAT activity was decreased by 68% in the initial study and to a lesser extent (−51%) in the latter study.

More recently, we demonstrated that oral administration of avasimibe to miniature pigs fed a fat- and cholesterol-containing diet significantly decreased the secretion of apoB containing lipoproteins into plasma [31]. Avasimibe (10–25 mg/kg/day) decreased the VLDL apoB pool size by 40 to 43% and the hepatic secretion of VLDL apoB into plasma by 38 to 41%. Hepatic microsomal ACAT activity was decreased by 51 to 68% with avasimibe treatment. ACAT inhibition by avasimibe decreased LDL pool size by 35 to 57%, largely due to a dose-dependent 25 to 63% reduction in the LDL apoB production rate.

In the same animal model, we demonstrated that the HMG-CoA reductase inhibitor, atorvastatin increases the fractional clearance rate of postprandial TRL [32]. Subsequent human studies in normolipidemic subjects [33] and postinfarction patients with combined hyperlipidemia [34] treated with atorvastatin were consistent with our findings. Taken together, these studies support the role of the miniature pig as an appropriate model to study human postprandial lipoprotein metabolism.

The studies herein were designed to test the hypothesis that the inhibition of cholesterol esterification by avasimibe modulates intestinal TRL metabolism, in vivo. The metabolic parameters of postprandial TRL in plasma with avasimibe treatment were determined from a previously described multi-compartmental model of TRL metabolism using kinetic analysis [32].

2. Design and methods

2.1. Animals and diets

Miniature pigs weighing 26.53±0.43 kg were obtained from a local supplier (Premier Quality Genetics Inc., West Lorne, Ontario). After being acclimatized for 1 week, animals were maintained on the experimental diet for 28 days before the postprandial studies. Pigs were studied in pairs, with each pair being same-sex littermates. Each animal received a 590 g ration of diet (B.W.S. Hog Grower, B-W Feed and Seed Ltd., New Hamburg, Canada) supplemented with lard, butter, and safflower oil (1:0.6:0.2) generating a final polyunsaturated: monounsaturated: saturated fatty acid ratio of 1:1:1. Cholesterol (Fisher Scientific, Ottawa, Ontario, Canada) was added to the diet to a final concentration of 0.1% (0.2 mg/kcal). This diet provided 34% calories from fat, 49% as carbohydrate and 17% as protein.

Pigs were studied in pairs with each pair being same sex litter mates. Five animals received the ACAT inhibitor, avasimibe (Pfizer) at a dose of 10 mg/kg/day and four animals, avasimibe at a dose of 25 mg/kg/day for 28 days prior to the postprandial studies. Avasimibe was placed in gelatin capsules and to ensure ingestion was administered by hand before the daily feeding. The nine control animals received a placebo capsule. The avasimibe was given at 9 AM each day after a 24 h fast.

Two weeks prior to the postprandial studies, an indwelling silicone elastomer (Silastic) catheter (1.96 mm internal diameter) was surgically implanted in an external jugular vein. Isoflurane USP (Abbott Laboratories Ltd., Montreal, Canada) was used as the anesthetic and ketamine USP (Vetrepharm Canada Inc.) as the preanesthetic. Catheters that were kept patent by filling with 7% EDTA-Na_2, allowed for blood sampling throughout each postprandial study in unrestrained, unanesthetized animals. The Animal Care Committee of the University of Western Ontario approved the experimental protocol.

2.2. Oral fat tolerance test

After a 24 h fast, pigs were fed the diet described above, in an amount calculated to provide 2 g of fat/kg body weight and either placebo or avasimibe. This test meal was supplemented with 50,000 IU of retinol (Vitamin A capsules USP, Novopharm Ltd., Toronto, Canada) and consumed within 10 min. The animals were not fed for the 12-h study but had free access to drinking water. As described [32], venous blood samples (20 mL) were drawn at intervals and

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