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In vivo efficacy of acyl CoA: Diacylglycerol acyltransferase (DGAT) 1 inhibition in rodent models of postprandial hyperlipidemia

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ABSTRACT

Postprandial serum triglyceride concentrations have recently been identified as a major, independent risk factor for future cardiovascular events. As a result, postprandial hyperlipidemia has emerged as a potential therapeutic target. The purpose of this study was two-fold. Firstly, to describe and characterize a standardized model of postprandial hyperlipidemia in multiple rodent species; and secondly, apply these rodent models to the evaluation of a novel class of pharmacologic agent; acyl CoA:diacylglycerol acyltransferase (DGAT) 1 inhibitors. Serum triglycerides were measured before and for 4 h after oral administration of a standardized volume of corn oil, to fasted C57BL/6, *ob*/*ob*, apoE$^{-/-}$ and CD-1 mice; Sprague–Dawley and JCR/LA-*cp* rats; and normolipidemic and hyperlipidemic hamsters. Intragastric administration of corn oil increased serum triglycerides in all animals evaluated, however the magnitude and time-course of the postprandial triglyceride excursion varied. The potent and selective DGAT-1 inhibitor A-922500 (0.03, 0.3 and 3 mg/kg, p.o.), dose-dependently attenuated the maximal postprandial rise in serum triglyceride concentrations in all species tested. At the highest dose of DGAT-1 inhibitor, the postprandial triglyceride response was abolished. This study provides a comprehensive characterization of the time-course of postprandial hyperlipidemia in rodents. In addition, the ability of DGAT-1 inhibitors to attenuate postprandial hyperlipidemia in multiple rodent models, including those that feature insulin resistance, is documented. Exaggerated postprandial hyperlipidemia is inherent to insulin-resistant states in humans and contributes to the substantially elevated cardiovascular risk observed in these patients. Therefore, by attenuating postprandial hyperlipidemia, DGAT-1 inhibition may represent a novel therapeutic approach to reduce cardiovascular risk.

1. Introduction

Serum triglyceride concentrations increase following ingestion of a fat containing meal resulting in postprandial hyperlipidemia. Peak serum triglyceride levels are observed within 2 to 4 h of fat consumption and then gradually return to baseline levels within approximately 10 h (Cohn et al., 1988). Zilversmit first proposed that the almost continuous exposure to these postprandial triglyceride-containing lipoproteins is the most significant cause of atherosclerosis, and he subsequently termed atherogenesis a "postprandial phenomenon" in the 1970s (Zilversmit, 1979). The recently published Copenhagen City Heart Study (Nordestgaard et al., 2007) and the Women's Health Study (Bansal et al., 2007) both corroborated this long-standing hypothesis by documenting postprandial serum triglycerides as a major, independent risk factor for future cardiovascular events in a fully adjusted analysis.

These epidemiological findings have intensified scientific interest in postprandial lipoproteins, and chylomicrons have remerged as a potential therapeutic target to inhibit atherogenesis (Stalenhoef and Watts, 2008). In fact, Redgrave recently advocated that postprandial dyslipidemia should become a focus of drug development (Redgrave, 2008). However, despite recent efforts to establish a standardized oral triglyceride tolerance test to evaluate postprandial lipid metabolism in the clinic (Ridker, 2008; van Oostrom et al., 2009; Warnick and Nakajima, 2008; Weiss et al., 2008), preclinical animal models of postprandial hyperlipidemia to facilitate drug discovery have not been well characterized. There have been sporadic reports on the use of an oral lipid challenge to produce postprandial hyperlipidemia in experimental animals, although methodologies, including the composition and dose of lipid, have been inconsistent (Buhman et al., 2002; Fujinami et al., 2001; Vine et al., 2007). Therefore, the first purpose of this study was to describe and characterize a standardized model of postprandial hyperlipidemia in multiple rodent species to allow evaluation of pharmacological modifiers of postprandial lipoprotein metabolism.

Dietary triglycerides are hydrolyzed in the small intestine by pancreatic lipase to monoacylglycerol and fatty acids, which are then

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absorbed by the enterocytes and recombined into triglycerides by a series of sequential esterification steps, the final of which is catalyzed by acyl CoA:diacylglycerol acyltransferase (DGAT). The re-synthesized triglycerides are then incorporated into chylomicrons and secreted into the circulation via the lymphatic system. DGAT-1 is one of two known DGAT enzymes (Cases et al., 1998). The highest levels of DGAT-1 expression are found in the small intestine (Yen et al., 2008). In addition, DGAT-1 knockout mice have dramatically reduced levels of intestinal triglyceride synthesis and chylomicron secretion following an oral lipid challenge (Buhman et al., 2002). Consequently, DGAT-1 represents a credible target for the treatment of postprandial hyperlipidemia. Therefore, the second purpose of this study was to determine the effect of a potent and selective DGAT-1 inhibitor on the standardized rodent models of postprandial hyperlipidemia described.

2. Methods

2.1. Animals and diets

All protocols were approved by the Abbott Laboratories Institutional Animal Care and Use Committee and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animals were housed under standard laboratory conditions with a 12 h light/dark cycle, in a temperature and humidity controlled room.

2.1.1. Mice

Male C57BL/6, leptin deficient (*ob/ob*) and apolipoprotein E knockout (apoE$^{-/-}$) mice all in a C57BL/6 background were obtained from The Jackson Laboratory (Bar Harbor, ME). Male CD1/ICR mice were obtained from Charles River Laboratories (Portage, MI). All mice were 5–9 weeks of age at study initiation and were fed a standard rodent diet (Harlan 2018) ad libitum and provided free access to water.

2.1.2. Rats

Male Sprague–Dawley and JCR:LA-*cp* rats, both obtained from Charles River Laboratories (Portage, MI), were 6–9 weeks of age at study initiation. All rats were provided ad libitum access to a standard rodent diet (Harlan 2018) and water.

2.1.3. Hamsters

Thirteen-week old Male Golden Syrian hamsters, obtained from Charles River Laboratories (Kingston, NY), were 100–150 g at the time this study. Hamsters were housed on a reversed 12-h light/dark cycle (7 pm–7 am) and were given ad libitum access to water and standard chow (Harlan 2018). Hyperlipidemia was induced in a separate group of hamsters by feeding a high fat diet (Purina no. 5001 with 11.5% corn oil, 11.5% coconut oil, 0.5% cholesterol, 0.25% deoxycholate, Dyets, Bethlehem, PA) for 14 days, with 10% fructose in the drinking water (Sigma, St. Louis, MO). This model has been previously reported to reliably induce dyslipidemia within 7 days, and serum lipids stabilize within 14 days (Wang et al., 2001).

2.2. Blood sampling

Mice were sacrificed via CO_2 inhalation and then 500 µl of blood was collected via cardiac puncture. Conscious rats were placed in a rodent restrainer and 500 µl of blood was collected from the tail vein. Hamsters were anesthetized in an induction chamber, using 4% isoflurane in oxygen, and 500 µl of blood was collected from the retro-orbital sinus. All blood samples were collected into a serum separator microtainer tube. Only one blood sample was collected from each mouse and hamster, while multiple samples could be drawn from each rat. Therefore the postprandial time-course of serum triglyceride levels represents blood samples taken from different groups of mice and hamsters at each time-point, whereas the time-course in rats is obtained from the same group of rats.

2.3. Serum lipid measurements

Serum triglycerides were measured in the clinical pathology laboratory at Abbott Laboratories on an Aeroset c8000 clinical chemistry analyzer (Abbott Laboratories, Abbott Park, IL) using photometric methods.

2.4. Oral triglyceride tolerance test

Mice (C57BL/6 J, ob/ob, apoE$^{-/-}$ and CD-1) and rats (SD and JCR:LA-*cp*) were fasted overnight for 16 h and hamsters (normolipidemic and dyslipidemic) were fasted for 4 h. All animals were fasted in new cages, with free access to water. At time zero ($t=0$), seven to ten animals from each group were bled for the measurement of baseline serum triglyceride levels. The remaining animals were then administered a corn oil bolus (6 ml/kg) via oral gavage. Seven to ten animals from each group were then bled at one ($t=1$), two ($t=2$) and three ($t=3$) h after the corn oil bolus for determination of serum triglyceride concentration. The peak postprandial response in serum triglyceride levels in JCR:LA-*cp* rats has previously been reported to occur 2 to 4 h following an oral lipid challenge (Vine et al., 2007). Therefore, to minimize the number of bleeds required and to ensure the peak response was captured, blood samples were collected in JCR:LA-*cp* rats two ($t=2$) and four ($t=4$) h after administration of corn oil.

2.5. Acute effect of DGAT-1 inhibition on postprandial hyperlipidemia

Mice (C57BL/6 J, ob/ob, apoE$^{-/-}$ and CD-1) and rats (SD and JCR:LA-*cp*) were fasted overnight for 16 h in new cages, with free access to water. One hourbefore corn oil administration ($t=-1$), 7–10 animals from each group were randomly assigned to receive either vehicle (20:80 v/v, polyethylene glycol: hydroxypropyl-β-cyclodextrin (10% w/v)), or DGAT-1 inhibitor A-922500 (Fig. 1) at 0.03, 0.3 or 3.0 mg/kg by oral gavage (6 ml/kg). A-922500 is a potent, selective and orally bioavailable DGAT-1 inhibitor exhibiting an IC_{50} value of 9 ηM and 22 ηM against human and mouse DGAT-1 respectively (Zhao et al., 2008). A-922500 demonstrates over 1000-fold selectivity over other acyltransferases including DGAT-2 ($IC_{50}=53$ µM) and the phylogenetic family members, ACAT-1 and ACAT-2 ($IC_{50}=296$ µM) (Zhao et al., 2008). The selectivity of a DGAT-1 inhibitor over DGAT-2 is not surprising given the enzymes only share 12% amino-acid sequence homology (Cases et al., 1998; Cases et al., 2001). One hour ($t=0$) after administration of vehicle or DGAT-1 inhibitor, all animals were given an oral bolus of corn oil (6 ml/kg). Serum triglyceride levels were then measured 2 h later ($t=2$), except in JCR/LA-*cp* rats where serum triglycerides were measured 4 h after corn oil administration ($t=4$). Seven to ten untreated animals provided a baseline serum triglyceride measurement. The effect of DGAT-1 inhibition on postprandial hyperlipidemia in hamsters was not evaluated as the increase in serum triglycerides observed after corn oil administration in both normolipidemic and hyperlipidemic hamsters was small in magnitude and quite variable.

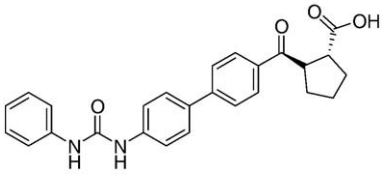

Fig. 1. A-922500: a potent and selective small molecule inhibitor of DGAT-1.