Diacylglycerol Acyltransferase 1 Inhibition Lowers Serum Triglycerides in the Zucker Fatty Rat and the Hyperlipidemic Hamster

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ABSTRACT

Acyl CoA/diacylglycerol acyltransferase (DGAT) 1 is one of two known DGAT enzymes that catalyze the final and only committed step in triglyceride biosynthesis. The purpose of this study was to test the hypothesis that chronic inhibition of DGAT-1 with a small-molecule inhibitor will reduce serum triglyceride concentrations in both genetic and diet-induced models of hypertriglyceridemia. Zucker fatty rats and diet-induced dyslipidemic hamsters were dosed orally with A-922500 (0.03, 0.3, and 3-mg/kg), a potent and selective DGAT-1 inhibitor, for 14 days. Serum triglycerides were significantly reduced by the 3 mg/kg dose of the DGAT-1 inhibitor in both the Zucker fatty rat (39%) and hyperlipidemic hamster (53%). These serum triglyceride changes were accompanied by significant reductions in free fatty acid levels by 32% in the Zucker fatty rat and 55% in the hyperlipidemic hamster. In addition, high-density lipoprotein-cholesterol was significantly increased (25%) in the Zucker fatty rat by A-922500 administered at 3 mg/kg. This study provides the first report that inhibition of DGAT-1, the final and only committed step of triglyceride synthesis, with a selective small-molecule inhibitor, significantly reduces serum triglyceride levels in both genetic and diet-induced animal models of hypertriglyceridemia. The results of this study support further investigation of DGAT-1 inhibition as a novel therapeutic approach to the treatment of hypertriglyceridemia in humans, and they suggest that inhibition of triglyceride synthesis may have more diverse beneficial effects on serum lipid profiles beyond triglyceride lowering.

Excessive accumulation of triglycerides in the blood and tissues is a central component of numerous pathophysiological conditions, including atherogenic dyslipidemia, obesity, insulin resistance, and hepatic steatosis. Therefore, inhibition of triglyceride synthesis has enormous therapeutic potential in the treatment of diverse metabolic disturbances, including hypertriglyceridemia. Two major pathways for triglyceride biosynthesis have been identified: the glycerol phosphate pathway (Kennedy, 1957) and the monoacylglycerol pathway (Kayden et al., 1967). The final and only committed step in the synthesis of triglycerides is common to both pathways and is catalyzed by acyl CoA/diacylglycerol acyltransferase (DGAT) enzymes (Yen et al., 2008).

DGAT-1 is one of two known DGAT enzymes (Cases et al., 1998) and seems to play a critical role in the pathogenesis of both obesity and insulin resistance in rodent models (Chen and Farese, 2005). For example, homozygous DGAT-1 knockout mice are resistant to the effects of diet-induced obesity and have an increased sensitivity to both insulin and leptin (Smith et al., 2000; Chen et al., 2002, 2003, 2004). In addition, these animals have decreased levels of tissue triglycerides, are resistant to weight gain and hepatic steatosis when fed a high-fat diet, and demonstrate increased energy expenditure (Smith et al., 2000; Chen et al., 2002, 2003). Mice with heterozygous deletion of DGAT-1 exhibit an intermediate phenotype (Smith et al., 2000). We have recently reported that small molecule inhibition of DGAT-1 recapitulates the major phenotypic characteristics of the knockout mice (Zhao et al., 2008). In that study, administration of a potent and selective DGAT-1 inhibitor produced sustained weight loss and significant reductions in hepatic triglyceride concentrations in diet-induced obesity mice (Zhao et al., 2008). However, the effect of DGAT-1 inhibition on serum triglyceride levels in animal models of hypertriglyceridemia has not yet been determined. The purpose of this study was to test the
hypothesis that chronic small-molecule inhibition of DGAT-1 will reduce serum triglyceride concentrations in both diet-induced (hyperlipidemic hamster) and genetic (Zucker fatty rat) models of hypertriglyceridemia.

Similar to humans, and unlike other rodents, the Golden Syrian hamster carries a substantial amount of serum cholesterol in the LDL fraction and is sensitive to dyslipidemic diets. Wang and colleagues reported a hamster model of diabetic dyslipidemia produced by a diet high in fat, cholesterol, and fructose (Wang et al., 2001). Marked increases in microsomal DGAT activity in the liver, intestines, muscle, and adipose tissue have been observed in this model and were predominantly attributed to increased DGAT-1 expression, although additional post-transcriptional regulation was not discounted (Casaschi et al., 2005). A selective DGAT-1 inhibitor provides a tool to discern the functional significance of these findings.

Zucker fatty rats incorporate a spontaneously occurring mutation in the leptin receptor resulting in a 10-fold reduction in the binding affinity for leptin and dramatically attenuated leptin signaling (Chua et al., 1996). The phenotype of the Zucker fatty rat includes components of the metabolic syndrome, including obesity, insulin resistance, and a dyslipidemia characterized by hypertriglyceridemia and hypercholesterolemia (Amy et al., 1988). Measurements of triglyceride biosynthetic enzyme activity in obese Zucker rats by Jamdar and Cao (1995) revealed that the entire process of esterification in the glycerol phosphate pathway is accelerated, contributing to the phenotype of this animal model. However, the relative contribution of DGAT-1 to this enhanced triglyceride synthesis remains unknown, because the individual DGAT genes had not been identified and cloned at the time of that study (Cases et al., 1998, 2001). Administration of a DGAT-1 inhibitor in the current study specifically investigated the role of DGAT-1 in the phenotype of Zucker fatty rats.

A-922500 (Abbott Laboratories, Abbott Park, IL) is a potent, selective, and orally bioavailable DGAT-1 inhibitor exhibiting IC_{50} values of 9 and 22 nM against human and mouse DGAT-1, respectively (Fig. 1). A-922500 demonstrates excellent selectivity over other acyltransferases, including DGAT-2 (IC_{50} = 53 μM) and the phylogenetic family members acyl coenzyme A cholesterol acyltransferase-1 and -2 (IC_{50} = 296 μM). The synthesis route and pharmacokinetic characteristics of A-922500 have been reported previously (Zhao et al., 2008). In the current study, we treated hypertriglyceridemic Zucker fatty rats and diet-induced dyslipidemic hamsters with three different oral doses of A-922500 for 14 days.

![Fig. 1. A-922500, a potent and selective small-molecule inhibitor of DGAT-1.](image)

### Materials and Methods

#### Animal Models and Diets

All protocols were approved by the 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. Thirteen-week-old male Golden Syrian hamsters (n = 40; Charles River Laboratories, Kingston, NY), initially weighing approximately 140 g, were housed individually in a temperature- and humidity-controlled room and allowed free access to standard rodent diet (2018; Harlan Teklad, Madison, WI) and water. Hamsters were acclimated to a reversed 12-h light/dark cycle (7:00 PM–7:00 AM). Hyperlipidemia was then induced by feeding a high-fat diet (Purina 5001 with 11.5% corn oil, 11.5% coconut oil, 0.5% cholesterol, and 0.25% deoxycholate; Dyets, Bethlehem, PA) for 14 days, with 10% fructose (Sigma-Aldrich, St. Louis, MO) in the drinking water. This model has been reported previously to reliably induce dyslipidemia within 7 days, and serum lipids stabilize within 14 days (Wang et al., 2001).

Ten-week-old male Zucker fatty rats (n = 32; Charles River Laboratories, Raleigh, NC), weighing between 270 and 330 g, were pair housed in a temperature- and humidity-controlled room with a 12-h light/dark cycle (7:00 AM–7:00 PM) and allowed free access to standard rodent diet (2018; Harlan Teklad) and water for the duration of the study.

#### Blood Sampling

After a 4-h fast, hamsters were anesthetized in an induction chamber, using 4% isoflurane in oxygen, and 500 μl of blood was collected into a serum separator Microtainer tube (BD Biosciences, San Jose, CA) from the retro-orbital sinus. Overnight fasted rats were placed in a rodent restrainer, and 500 μl of blood was collected from the tail vein.

#### Serum Lipid Measurements

Serum triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, and free fatty acid concentrations were measured in the clinical pathology laboratory at Abbott Laboratories on an Aeroset e8000 clinical chemistry analyzer (Abbott Laboratories).

#### Experimental Protocol

After collection of baseline lipid profiles, hyperlipidemic hamsters (n = 10/group) and Zucker fatty rats (n = 8/group) were administered vehicle [20:80 (v/v), polyethylene glycol/hydroxypropyl-β-cyclodextrin (10% w/v)] (Sigma-Aldrich) or DGAT-1 inhibitor A-922500 at 0.03, 0.3, and 3-mg/kg, once daily by oral gavage. The dosing volume was 5 ml/kg. Serum lipid profiles were then measured 3 h after the dose on day 7 (data not shown) and day 14. Hamsters continued to be fed a high-fat diet with 10% fructose in the drinking water throughout the treatment period. Zucker fatty rats remained on standard rodent diet throughout the study.

#### Statistical Analysis

The effect of vehicle or DGAT-1 inhibition on serum lipid parameters and body weight was assessed by a two-way mixed design (time as a repeated measure) ANOVA, with post hoc testing performed comparing day 14 to baseline using Bonferroni's procedure to correct for multiple comparisons (Prism 4.0; GraphPad Software Inc., San Diego, CA). The change in serum lipid parameter and body weight over the 14-day treatment with DGAT-1 inhibitor was compared with the change produced by vehicle administration using a one-way ANOVA with Dunnett’s multiple comparisons. Before the application of parametric tests, the data were assessed with the D’Agostino and Pearson omnibus normality test (Prism 4.0). Data that were not normally distributed (p < 0.05) were log-transformed to generate a normal distribution before parametric testing (Prism 4.0). A p value of <0.05 was considered significant. All results are presented as mean ± S.E.

### Results

#### Diet-Induced Dyslipidemia in Hamsters

The effect of 2 weeks of feeding a hyperlipidemic diet to hamsters is shown in Fig. 2. A high-fat diet with 10% fructose in the drinking
water significantly increased serum triglycerides (4.7-fold), total cholesterol (3.0-fold), LDL-cholesterol (5.1-fold), HDL-cholesterol (1.6-fold), and FFA levels (1.5-fold) and produced a 6.3% increase in body weight. This mixed dyslipidemia, including elevated serum HDL concentrations, has been reported consistently in hamster models of diet-induced hyperlipidemia (Woollett et al., 1997; Rémillard et al., 2001; Wang et al., 2001; Casaschi et al., 2005; Bilz et al., 2006).

Triglyceride Lowering by DGAT-1 Inhibition in Dyslipidemic Hamsters. The effect of administration of vehicle or a DGAT-1 inhibitor for 14 days on body weight and serum lipid parameters in dyslipidemic hamsters is shown in Fig. 3. Vehicle treatment had no significant effect on body weight or serum lipid profile. DGAT-1 inhibitor A-922500 reduced serum triglyceride levels from baseline at all doses tested; however, this was only statistically significant at the 3 mg/kg dose, which lowered serum triglycerides by 53%. Similarly, the 3 mg/kg dose of A-922500 significantly reduced serum FFA concentrations by 55% and total cholesterol by 25%. DGAT-1 inhibition had no significant effect on body weight at any dose tested. Although A-922500 did not significantly affect LDL-cholesterol or HDL-cholesterol individually, the serum LDL/HDL-cholesterol ratio was significantly improved by A-922500 at 0.3 and 3 mg/kg.

Triglyceride Lowering by DGAT-1 Inhibition in Zucker Fatty Rats. The effect of administration of vehicle or a DGAT-1 inhibitor for 14 days on body weight and serum lipid parameters in Zucker fatty rats is shown in Fig. 4. Similar to the dyslipidemic hamster, treatment with 3 mg/kg A-922500 significantly reduced serum triglyceride concentrations (39%). FFA levels significantly increased over the 14-day period in vehicle-treated animals. This increase was inhibited in a dose-dependent manner by A-922500 such that FFA concentrations were 32% lower after 14 days of treatment with the DGAT-1 inhibitor at 3 mg/kg, compared with the vehicle group (p < 0.05). HDL-cholesterol was significantly increased from baseline levels by A-922500 at 0.3 and 3 mg/kg; however, this was only significantly increased compared with vehicle at the 3 mg/kg dose. Body weight significantly increased over the 2-week period in vehicle-treated rats, and this was not affected by A-922500. LDL-cholesterol was significantly reduced in the vehicle treated group. DGAT-1 inhibition did not further reduce LDL-cholesterol and had no effect on total cholesterol.

Discussion

We report for the first time that inhibition of the final and only committed step of triglyceride synthesis with a selective small-molecule inhibitor of DGAT-1 significantly reduces serum triglyceride levels in both genetic and diet-induced animal models of hypertriglyceridemia. The results of this study support further investigation of DGAT-1 as a novel therapeutic target for the treatment of hypertriglyceridemia in humans. In addition to reductions in serum triglyceride concentrations, DGAT-1 inhibition produced beneficial effects on other blood lipids in the current study, including reductions in both FFA levels and total cholesterol and increases in
HDL-cholesterol. The combined improvements in serum lipid profiles produced by DGAT-1 inhibition offer therapeutic potential for the reduction of atherosclerotic cardiovascular risk.

Currently available pharmaceutical agents that target specific components of FFA and triglyceride metabolism, such as niacin and fibrates, dramatically reduce serum triglyceride concentrations and are among the most potent compounds at increasing HDL-cholesterol levels (Joy and Hegele, 2008). Therefore, it was not surprising that the serum triglyceride reductions produced by DGAT-1 inhibition in this study, at least in the Zucker fatty rat, were accompanied by significant increases in HDL-cholesterol. Elevations in triglyceride-rich lipoproteins actively promote core lipid exchange between lipoproteins to produce HDL particles enriched in triglyceride (Rashid et al., 2002). Extensive evidence indicates that triglyceride-enriched HDL is catabolized more rapidly than native HDL, ultimately reducing HDL-cholesterol concentrations (Lamarche et al., 1999a,b). Therefore, should inhibition of triglyceride synthesis with DGAT-1 inhibitors prove effective at lowering serum triglycerides in humans, additional beneficial effects on serum lipids, including elevations in HDL-cholesterol, are anticipated. A-922500 also tended to increase HDL-cholesterol and reduce LDL-cholesterol in the hyperlipidemic hamster, but these changes were not statistically significant. However, when these effects were combined to generate the LDL/HDL ratio, which continues to serve as a valuable and standard tool to evaluate cardiovascular risk (Fernandez and Webb, 2008), significant improvements in the serum cholesterol profile were observed.

DGAT-1 inhibitors may produce more diverse metabolic improvements beyond lipid lowering, which would make them ideally suited for the treatment of the constellation of cardiovascular risk factors in the metabolic syndrome (Zammit et al., 2008). Insulin resistance, a hallmark of the metabolic syndrome, seems to be a particularly potent driver of atherosclerosis and premature cardiovascular death in these patients (Razani et al., 2008). The current approach to the prevention of cardiovascular disease in insulin resistance relies on risk factor modification, such as the treatment of hypertension and hyperlipidemia, rather than directly targeting insulin sensitivity (Razani et al., 2008). In addition, the recent emergence of safety concerns surrounding the initially promising insulin-sensitizing thiazolidinedione
agents has produced uncertainty in the therapeutic management of insulin resistance (Erdmann and Wilcox, 2008). DGAT-1 knockout mice have an increased sensitivity to insulin, and homozygous deletion of the DGAT-1 gene in the severely insulin resistant agouti yellow mice significantly improves insulin sensitivity (Chen et al., 2002). Chronic elevations in FFAs cause insulin resistance in skeletal muscle and the liver by interfering with insulin signaling and also possibly impair pancreatic β-cell function through lipotoxicity (Wilding, 2007). In the current study, DGAT-1 inhibition significantly reduced FFA levels in the hyperlipidemic hamster and prevented the increase in FFA concentrations observed in the vehicle-treated Zucker rat over the 2-week period. An increase in serum FFA concentrations occurs 2 weeks before the onset of diabetes in Zucker diabetic rats, and it has been reported that this is an initiating factor in the genesis of diabetes in this model (Unger, 1995). Therefore, it is appealing to speculate that the chronic modulation of FFA levels produced by DGAT-1 inhibition here will improve insulin sensitivity. However, this remains to be determined and warrants further investigation. The mechanism by which DGAT-1 inhibition reduces serum FFA concentrations is not apparent at this time, although studies in DGAT-1 knockout mice reveal both impairments in lipid absorption (Buhman et al., 2002) and a 20% increase in metabolic rate (Smith et al., 2000). It would also be of interest to assess the molecular composition of serum FFAs in future studies, because microdetermination of FAs may provide for stratification of cardiovascular risk (Rupp et al., 2006), and DGAT-1 deficiency in mice has been shown to alter tissue fatty acid composition (Buhman et al., 2002; Chen et al., 2002).

The highest levels of DGAT-1 expression are found in the small intestine (Yen et al., 2008). DGAT-1 is also expressed in other triglyceride-producing tissues, including adipose tissue and the liver, albeit at lower levels (Yen et al., 2008). Conversely, the expression levels of DGAT-2 are highest in adipose tissue and liver, with significantly lower expression in the small intestine (Yen et al., 2008). DGAT-1 knockout mice have dramatically reduced levels of intestinal triglyceride synthesis and chylomicron secretion after a lipid challenge (Buhman et al., 2002), whereas hepatic triglyceride secretion is unaffected (Liu et al., 2008). Combined, these data suggest that DGAT-1 inhibitors are likely to exert major pharmacologic actions in the gastrointestinal tract. Indeed, we have reported that DGAT-1 inhibitor A-922500 prevents postprandial elevations in serum triglyceride levels in mice (Zhao et al., 2008). Recent evidence has advanced the concept that intestinal lipoprotein overproduction is a major contributor to both the fasting and postprandial dyslipidemia observed in insulin-resistant states (Adeli and Lewis, 2008; Duez et al., 2008). Significant oversecretion of apolipoprotein B48-containing lipoproteins from the intestine has been documented in both fasting and postprandial states in hyperlipidemic hamsters (Haidari et al., 2002). Furthermore, postprandial hyperlipidemia after an oral triglyceride tolerance test is exacerbated in Zucker diabetic fatty rats compared with lean controls by approximately 8-fold (Fujinami et al., 2001). Therefore excessive intestinal triglyceride synthesis contributes to the dyslipidemia of both models examined in this study. The documented action of DGAT-1 inhibitors to abolish postprandial hyperlipidemia probably contributes to the lipid-lowering effects seen in the current study.

Traditionally, pharmacotherapy in dyslipidemic patients has focused on reducing LDL-cholesterol. Recent develop-
ments have lead numerous investigators to question the value of reducing LDL-cholesterol beyond what is already currently achieved and advocated that mechanisms other than LDL-cholesterol lowering must now be the priority for new therapeutic approaches to the treatment of dyslipidemia (Grundy, 2008). Inhibition of triglyceride synthesis with a DGAT-1 inhibitor offers a novel therapeutic approach to dyslipidemia. In addition to the beneficial effects on serum lipids demonstrated in the current study, DGAT-1 inhibitors have enormous therapeutic potential to reduce cardiovascular risk by exerting beneficial metabolic effects, including insulin sensitization as reported in DGAT-1 knockout mice.

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References


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