PRD125, a Potent and Selective Inhibitor of Sterol O-Acyltransferase 2 Markedly Reduces Hepatic Cholesteryl Ester Accumulation and Improves Liver Function in Lysosomal Acid Lipase-Deficient Mice

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Received June 24, 2015; accepted August 14, 2015

ABSTRACT

In most organs, the bulk of cholesterol is unesterified, although nearly all possess a varying capability of esterifying cholesterol through the action of either sterol O-acyltransferase (SOAT) 1 or, in the case of hepatocytes and enterocytes, SOAT2. Esterified cholesterol (EC) carried in plasma lipoproteins is hydrolyzed by lysosomal acid lipase (LAL) when they are cleared from the circulation. Loss-of-function mutations in LIPA, the gene that encodes LAL, result in Wolman disease or cholesteryl ester storage disease (CESD). Hepatomegaly and a massive increase in tissue EC levels are hallmark features of both disorders. While storage disease (CESD). Hepatomegaly and a massive increase in tissue EC levels are hallmark features of both disorders. While

Introduction

In healthy young adult mammals ranging from mice to primates, the pool of cholesterol in the whole animal averages about 2200 mg/kg body weight, with this sterol originating in varying degrees from endogenous and exogenous sources (Grundy, 1983; Turley and Dietschy, 1988; Dietschy and Turley, 2004). Ordinarily, in nearly all the major organ systems, the bulk of this cholesterol is unesterified, (d’Hollander and Chevallier, 1969). Exceptions include the adrenal glands and plasma (Goodman, 1965; d’ Hollander and Chevallier, 1969). In some organs, most notably the liver, the esterified cholesterol (EC) content can vary over a wide range in response to shifts in the dietary intake of cholesterol or specific fatty acids (Rudel et al., 1997; Xie et al., 2002; Turley et al., 2010).

Most dietary cholesterol is unesterified (Tso, 1994). Even if this was not the case, none of the esterified cholesterol present in tissues originates from external sources because pancreatic cholesterol esterase hydrolyzes cholesteryl esters present in foodstuff (Tso, 1994). Moreover, esterified cholesterol is poorly absorbed (Goodman, 1965). Several organs are capable of generating esterified cholesterol through the action of either sterol O-acyltransferase (SOAT) 1 (also known as acylcoenzyme A cholesterol O-acyltransferase 1), which is present in steroidogenic tissues, kidneys, sebaceous glands, and macrophages, or SOAT2 (acyl-coenzyme A cholesterol O-acyltransferase 2), which is expressed in hepatocytes and enterocytes (Cases et al., 1998; Lee et al., 2000; Parini et al., 2004). In the plasma compartment, cholesterol esterification is facilitated by lecithin cholesterol acyltransferase (Rouset et al., 2011). The roles that both SOAT1 and SOAT2 play in generating cholesteryl esters, and therefore in the pathogenesis

ABBREVIATIONS: ALT, alanine transaminase; CESD, cholesteryl ester storage disease; EC, esterified cholesterol; LAL, lysosomal acid lipase; LDL, low density lipoprotein; PRD125, 1,11-O-o-methylbenzylidene-7-O-p-cyanobenzoyl-1,7,11-trideacetylpipipropene A; SOAT, sterol O-acyltransferase; TAG, triacylglycerol; UC, unesterified cholesterol; VLDL, very low density lipoprotein; WD, Wolman disease.
of atherosclerosis, have made these enzymes, particularly SOAT2, attractive targets for pharmacological intervention (Lada et al., 2004; Rudel et al., 2005; Chang et al., 2006; Farese, 2006). This has led to the identification of a new group of compounds that are selective inhibitors of SOAT2 (Ohshiro et al., 2011; Ohtawa et al., 2013). Prior to the discovery that there are two sterol O-acyltransferases, decades of research into the development of various classes of SOAT inhibitors yielded a number of compounds that markedly suppressed cholesterol esterification in vitro and in animal models. Clinical trials were carried out to evaluate the efficacy of two of these compounds, avasimibe and pactimibe, in slowing the progression of coronary atherosclerosis. In neither case was the outcome favorable (Tardif et al., 2004; Nissen et al., 2006).

Although the major focus on EC accumulation in disease centers on its involvement in arterial plaque formation (Peng et al., 2000), there are a number of rare disorders that are characterized by elevated tissue EC content (Goodman, 1965). These include Tangier disease, Wolman disease (WD), and cholesteryl ester storage disease (CESD) (Remaley et al., 1997; Grabowski and Du at the Children Hospital Research Foundation in Cincinnati, OH (Du et al., 1998, 2001). All mice were of the Friend leukemia virus B/N strain (FVB/N). Litters were genotyped at 18–20 days and weaned at 21 days. For all studies, the diet was used as a low fat rodent chow formulation (Teklad No. 7001; Harlan Laboratories, Madison, WI), with an inherent cholesterol content of 0.02% w/w (0.2 mg cholesterol/g diet). The development of PRD125 was carried out at the Graduate School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan. Its structure is shown in Fig. 1. PRD125 is a derivative of pyripyropene A (hence the prefix PRD), the first compound to exhibit strong and selective inhibitory action toward SOAT2 (Ohshiro et al., 2011). Its inhibitory activity toward SOAT1 and SOAT2 and isozyme selectivity were reported before the name PRD125 was applied. Initially, it was called compound 7q (Ohtawa et al., 2013) and was reported to have a selectivity index toward SOAT2 versus SOAT1 that was >6161. PRD125 was added directly to the meal (powder) form of this diet in the proportion of 62.5 mg of compound per 1000 g of chow. Initially, the compound was incorporated into a small amount of chow using a mortar and pestle. The resulting premix was then dispensed thoroughly in the remaining chow using a food mixer (Hobart Corp., Troy, OH). This formulation provided the mice with an approximate dose of PRD125 of 10 mg/day per kg body weight (bw) based on a daily food intake of about 160 g/day per kg bw. This figure was obtained from measurements of the food intake of three Lal−/− and three Lal+/− mice, all housed individually, over 4 consecutive days, starting when they were 40 days old. An estimated daily spillage of 10% was used in calculating their daily intake, which did not vary as a function of genotype.

Although the PRD125 feeding experiments were carried out in male mice only, the decisions regarding the age of when to start treatment and also the length of the treatment period were based to a considerable degree on metabolic data obtained from female Lal−/− mice at different stages of disease progression (21, 49–51, and 140–142 days of age). In all cases, those mice had been fed only the basal chow diet from the time of weaning to the day of study. For the PRD125 experiment, it was decided that treatment would start on the day of weaning (21 days) and continue until the mice were 52 or 53 days of age. Matching groups of Lal+/− and Lal−/− mice were given the chow diet alone, whereas corresponding groups were fed the diet containing PRD125. These mice were group housed up until about 1 week before study. They were then housed individually to facilitate stool collection over a 4-day period. In both studies, all animals had unrestricted access to their respective diet and water and were in the fed state at the time of study. All studies were approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center.

**Organ Resection and Processing.** The mice were anesthetized with isoflurane [2-chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane] and exsanguinated from the vena cava into a heparinized syringe. Plasma was obtained immediately thereafter and used for measurements of transaminase activity and total cholesterol concentration. The liver and whole small intestine were excised, and the latter was cut into eight segments that were perfused with saline from a syringe...
fitted with a blunt 16 G needle. After blotting on filter paper, the weight of all sections combined was recorded. The liver was also rinsed with saline and blotted before being weighed. The whole small intestine and several aliquots of liver were each added to about 30 ml of chloroform/methanol (2:1 v/v). The residual carcass was weighed and added to 200 ml of ethanolic potassium hydroxide.

**Hepatic, Intestinal, Whole Body, and Plasma Cholesterol Measurements.** For the liver and small intestine, the concentrations of unesterified and esterified cholesterol were measured using a combination of column and gas chromatography as described (Turley et al., 2010). These data were expressed as mg/g wet weight of tissue. The unesterified and esterified concentrations were summed to give the total cholesterol concentration (mg/g), which in turn was multiplied by the respective liver or small intestine weight to yield the whole organ cholesterol content (mg/organ). The total cholesterol concentration of the residual carcass (mg/g) was multiplied by its entire weight to obtain the whole carcass cholesterol content. The summation of these values for the liver, small intestine, and carcass yielded the whole body cholesterol content, expressed as mg/animal. Aliquots of plasma were saponified in 5 ml of ethanolic KOH, and the yield of the whole body cholesterol content, expressed as mg/organ.

**Fecal Neutral Sterol Excretion.** Rates of fecal neutral sterol excretion were determined on a 3-7 day stool collection. Additional details for the neutral sterol assay using gas chromatography were described earlier (Schwarz et al., 1998). The dominant neutral sterol was unmetabolized cholesterol, together with small amounts of coprostanol, epicoprostanol, and cholestanol. The rate of neutral sterol excretion was expressed as the equivalent mass of cholesterol appearing as neutral sterols per animal per day per 100 g bw.

**Relative mRNA Expression Analysis.** Aliquots of liver were quickly frozen in liquid nitrogen. mRNA levels were measured using a Bio-Rad CFX96 real-time polymerase chain reaction detection system (Hercules, CA). The primer sequences used to measure RNA expression included: cyclophilin, and the values for each mouse were then expressed relative to those obtained for their matching untreated controls, which, in each case, were arbitrarily set at 1.0.

**Analysis of Data.** Values are means ± S.E.M. for the specified number of animals. GraphPad Prism software, version 6.02 (San Diego, CA), was used to perform all statistical analyses. Depending on the design of each experiment, differences between mean values were tested for statistical significance (P≤0.05) by an unpaired two-tailed Student’s t test or a two-way analysis of variance, with genotype and treatment as the variables.

**Results**

**Lal**/−−** Mice Exhibit a Marked Increase in Hepatic Esterified Cholesterol Concentrations at an Early Age.** The data in Table 1 demonstrate multiple striking differences between Lal−/− mice and their Lal+/+ littersmates, starting at the time of weaning (21 days) and at early (49–51 days) and midadulthood (140–142 days). Genotypic differences in liver weights, already apparent at weaning, became highly pronounced as the mice aged. A very distinctive feature of the Lal−/− mice was their dramatic increase in hepatic EC concentration (mg/g), even at the time of weaning. In contrast, hepatic unesterified cholesterol (UC) concentrations in the mutants were elevated only moderately. The increases in both liver mass and hepatic EC concentration resulted in a whole liver cholesterol content (mg/organ) in the Lal−/− mice that was 12.2, 53.1, and 99-fold more than in their matching Lal+/+ controls at 21, 49–51, and 140–142 days, respectively. The genotypic differences in liver triacylglycerol (TAG) concentrations and contents generally followed those for esterified cholesterol, but were not as pronounced. The profound increases in hepatic TAG and especially EC levels in this mouse model are reflective of what has been described for the livers in a limited number of CESD patients (Sloan and Fredrickson, 1972; Hoeg et al., 1984; Todoroki et al., 2000). In the 49–51 and 140–142 day old Lal−/− mice, plasma ALT activities were elevated 8.1- and 22-fold, respectively. Based on the data in Table 1, it was decided to start PRD125 treatment on the fourth day the mice were weaned (21 days) and to continue it for 29–32 days.

**PRD125 Treatment Markedly Lowered the Esterified Cholesterol Concentration in the Small Intestine of Lal−/− Mice.** The final body weights of the Lal−/− and Lal+/+ mice given PRD125 were not different than those of the littermates of the same genotype that were fed the basal diet alone (Fig. 2A). Small intestine weights did not vary as a function of either genotype or treatment (Fig. 2B). Consistent with previous findings, the concentration of EC in the small intestine of the Lal−/− mice was very low, representing only about 4% of the total cholesterol concentration. There was a trend (P>0.05) to an even lower level when these mice were given PRD125 (Fig. 2C). In marked contrast, the intestinal EC level in the Lal−/− mice on chow only was elevated nearly 22-fold. Treatment with PRD125 blunted this increase by 50% (Fig. 2C). Apart from a marginal increase in the treated Lal−/− mice, the unesterified cholesterol concentrations were about the same in the four groups of mice (Fig. 2D).

**PRD125 Treatment Decreased Liver Mass and Affected a Pronounced Reduction in Hepatic Esterified Cholesterol Concentration in Lal−/− Mice.** The data for multiple parameters relating to the liver in the same groups of mice that the small intestines were taken from are shown in Fig. 3. In the treated Lal−/− mice, the absolute and relative liver weights were each 28% lower than those in their matching untreated Lal−/− littersmates (Fig. 3, A and B, respectively). The contraction in liver mass was accompanied by a 59% reduction in the UC concentration (Fig. 3C). The parameter that changed most in the treated mutants was whole liver cholesterol content, which contracted by 68% (Fig. 3E). Hepatic TAG concentrations were unchanged by PRD125 treatment in mice of either genotype. In the case of the mutants, these values were 21.1 ± 1.9 mg/g without treatment and 23.7 ± 3.5 mg/g with treatment. Hence, the modest reduction (P>0.05) in whole liver TAG content in the treated mutants (Fig. 3F) was accounted for fully by the contraction in liver mass in these mice (Fig. 3A).

**Actions of PRD125 in the Liver and Small Intestine Resulted in a Marked Reduction in Whole Body Cholesterol Content in Lal−/− Mice.** The next set of data broadly defines cholesterol metabolism in the animal as a whole. A key parameter in determining cholesterol turnover...
is the rate of fecal neutral excretion, which is a function of stool output and the concentration of neutral sterols per gram of stool. No genotypic or treatment-related changes in stool output were evident (Fig. 4A). A documented feature of LAL-deficient mice is an increased rate of fecal neutral sterol excretion (Aqul et al., 2014). This was evident from a comparison of the data for the chow-fed mutants versus wild-types in the current studies (Fig. 4B). PRD125 treatment clearly raised fecal sterol loss in the chow-fed mutants versus wild-types in the current studies (Aqul et al., 2014). This was evident from a comparison of the data for the treated mutants with those previously reported for LAL-deficient mice given PRD125. Accompanying these positive changes were decisive reductions in the plasma activities of ALT (Fig. 6A) and aspartate aminotransferase (Fig. 6B). It is noteworthy that the remarkable improvements in these indices of liver health occurred despite there being no reduction in hepatic TAG concentrations.

**Discussion**

The efficacy of PRD125 in blunting the continual expansion of the intestinal and hepatic pools of EC in LAL−/− mice is best illustrated by comparing the data for the treated mutants with those previously reported for LAL−/−Soat2−/− mice (Lopez et al., 2014). In those studies, the hepatic EC concentrations in LAL−/− mice is best illustrated by comparing the data for the treated mutants with those previously reported for LAL−/−Soat2−/− mice (Lopez et al., 2014). The data in Fig. 5 illustrate the dramatic reduction in the expression levels of mRNA for six genes that together reflect parameters, such as inflammation and macrophage presence, in the LAL-deficient mice given PRD125. Accompanying these positive changes were decisive reductions in the plasma activities of ALT (Fig. 6A) and aspartate aminotransferase (Fig. 6B). It is noteworthy that the remarkable improvements in these indices of liver health occurred despite there being no reduction in hepatic TAG concentrations.

**TABLE 1**

Characteristics of lysosomal acid lipase-deficient female mice at different stages of disease progression.

Data are presented as mean ± S.E.M. of values in four mice per group. All mice were weaned at 21 days of age and thereafter fed a basal rodent chow diet ad libitum. They were studied in the fed state.

<table>
<thead>
<tr>
<th>Age</th>
<th>Lal+/+</th>
<th>Lal−/−</th>
<th>Lal+/+</th>
<th>Lal−/−</th>
<th>Lal−/−</th>
<th>Lal−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>10.6±0.6</td>
<td>10.3±0.4</td>
<td>20.8±0.3</td>
<td>20.8±0.5</td>
<td>26.1±0.5</td>
<td>24.2±1.1</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>0.53±0.04</td>
<td>0.64±0.02</td>
<td>1.07±0.04</td>
<td>2.32±0.10*</td>
<td>1.08±0.04</td>
<td>4.93±0.26*</td>
</tr>
<tr>
<td>Hepatic esterified cholesterol concentration (mg/g)</td>
<td>1.0±0.3</td>
<td>30.0±0.6*</td>
<td>0.4±0.1</td>
<td>56.3±3.8*</td>
<td>0.8±0.1</td>
<td>53.7±2.3*</td>
</tr>
<tr>
<td>Hepatic unesterified cholesterol concentration (mg/g)</td>
<td>2.1±0.1</td>
<td>2.7±0.1*</td>
<td>2.0±0.1</td>
<td>3.2±0.1*</td>
<td>2.0±0.1</td>
<td>6.8±0.5*</td>
</tr>
<tr>
<td>Whole-liver cholesterol content (mg/organ)</td>
<td>1.7±0.2</td>
<td>20.8±1.0*</td>
<td>2.6±0.1</td>
<td>138.1±11.3*</td>
<td>3.0±0.2</td>
<td>297±10.7*</td>
</tr>
<tr>
<td>Hepatic triacylglycerol concentration (mg/g)</td>
<td>7.2±0.2</td>
<td>10.2±1.8</td>
<td>6.0±0.7</td>
<td>14.6±0.6*</td>
<td>12.6±1.5</td>
<td>19.4±0.8*</td>
</tr>
<tr>
<td>Whole-liver triacylglycerol content (mg/organ)</td>
<td>3.9±1.1</td>
<td>6.5±1.3</td>
<td>6.5±0.8</td>
<td>40.0±2.8*</td>
<td>13.6±1.9</td>
<td>97.4±10.6*</td>
</tr>
<tr>
<td>Plasma ALT activity (units/l)</td>
<td>32±2</td>
<td>39±5</td>
<td>33±2</td>
<td>268±37*</td>
<td>28±4</td>
<td>617±97*</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with corresponding Lal+/+ mice of the same age.
the \( \text{Lal}^{-/-} : \text{Soat}2^{-/-} \) mice versus their \( \text{Lal}^{-/-} : \text{Soat}2^{+/+} \) littermates, all at 52 days of age, were 15.2 versus 54.3 mg/g, respectively. The corresponding values for LAL mutants given PRD125 versus their untreated \( \text{Lal}^{-/-} \) mice controls were 23.4 and 56.5 mg/g, respectively (Fig. 3C). In the \( \text{Lal}^{-/-} \) mice given PRD125, liver mass was 28% less than that in the untreated \( \text{Lal}^{-/-} \) mice (Fig. 3A). This marked contraction in liver weight was comparable to that manifested in the \( \text{Lal}^{-/-} : \text{Soat}2^{-/-} \) mice (34%) (Lopez et al., 2014). From these sets of data, it can be calculated that the percentage reduction in whole liver cholesterol content (mg/organ) in the PRD125-treated \( \text{Lal}^{-/-} \) mice (68%) (Fig. 3E) was pronounced, although not as dramatic as that seen in the \( \text{Lal}^{-/-} : \text{Soat}2^{-/-} \) mice (80%). It is not yet known whether a higher dose of PRD125 would reduce hepatic EC content even further.

The mechanism(s) through which the remarkable cholesterol-lowering effects of SOAT2 deficiency or suppression are articulated in this CESD mouse model relate to the key role that this enzyme plays in determining the EC content of specific types of lipoprotein particles generated within the small intestine and liver. When SOAT2 in the small intestine is either absent or suppressed, this would not only contribute to a diminution of the EC concentration in the small intestine as a whole, but would also likely reduce the EC content of chylomicrons that are assembled in the intestine and ultimately cleared by the liver (Cooper, 1997). Such a reduced delivery of intestinal cholesterol to the liver would mimic the actions of the selective intestinal cholesterol absorption inhibitor ezetimibe in the \( \text{Lal}^{-/-} \) mouse (Chuang et al., 2014). Thus, to some extent, the pronounced fall in EC levels in the livers of the \( \text{Lal}^{-/-} : \text{Soat}2^{-/-} \) mice and PRD125-treated \( \text{Lal}^{-/-} \) mice is the product of a reduction in net cholesterol delivery from the small intestine to the liver. Clearance by the liver of chylomicron remnants with a reduced EC content would culminate in less EC becoming sequestered in the lysosomal compartment of hepatocytes and other cell types in the liver.

Ultimately, however, it is likely that the beneficial effect of suppressing or deleting SOAT2 activity arises primarily from the role that this enzyme plays in the formation of EC in the liver, and its subsequent incorporation into nascent very low density lipoproteins (VLDLs). This conclusion stems from a plethora of studies in various in vitro systems and animal models, particularly mouse models with liver- or intestine-specific deletion of SOAT2 (Zhang et al., 2012). The secretion from the liver of VLDL with a reduced EC content would potentially lower the amount of EC carried in mature LDL particles and VLDL remnants that are subsequently cleared from the circulation primarily by the liver, but also by the small intestine and other organs (Osono et al., 1995). In the
case of the liver, the clearance, not only of LDL and related particles with a reduced EC content, but also, as already noted, of chylomicron remnants containing diminished amounts of EC would result in a marked fall in the mass of EC sequestered in the lysosomes. Although tissue EC and UC levels were not determined in organs other than the liver and small intestine in either the PRD125-treated Lal\(^{-/-}\) mice or previously in the Lal\(^{-/-}\);Soat2\(^{-/-}\) mice, one might anticipate a reduction in EC content given that most extrahepatic organs take up some LDL from the circulation (Osono et al., 1995).

Several additional points relating to the data for the small intestine and also the rates of fecal neutral sterol excretion warrant discussion. Although hepatomegaly is pronounced in Lal\(^{-/-}\) mice by early adulthood, the mass of their small intestine has not changed significantly at that time. However, there are already marked histologic changes and increased levels of EC, both of which become very pronounced as disease progresses (Du et al., 2001; Aqul et al., 2014). The fall in intestinal EC concentration in the LAL mutants given PRD125 (Fig. 2) was comparable to that manifested in the Lal\(^{-/-}\);Soat2\(^{-/-}\) mice (Lopez et al., 2014). In interpreting data for intestinal cholesterol levels, one must take into account the fact that the mucosal surface is constantly undergoing renewal. In mice, the lifespan of the absorptive cells is only 2–3 days (Lipkin, 1981). If this turnover did not occur, the intestinal EC levels found in Lal\(^{-/-}\) mice would likely be even more elevated than shown here and in previous publications (Du et al., 2001; Aqul et al., 2014). Nevertheless, based on the present findings, the EC levels in either Lal\(^{-/-}\);Soat2\(^{-/-}\) mice or PRD125-treated LAL mutants would still be expected to be much lower than in unmanipulated Lal\(^{-/-}\) mice of the same age.

In the Lal\(^{+/+}\) mice given PRD125, there was a clear elevation in the rate of fecal neutral sterol excretion. This suggests either a marked inhibition of cholesterol absorption and/or an increased delivery of biliary cholesterol into the lumen as a consequence of SOAT2 inhibition in hepatocytes. As found previously, untreated Lal\(^{-/-}\) mice manifest higher rates of sterol excretion (Aqul et al., 2014). In this case, the increase in fecal sterol loss might reflect either a higher cholesterol content of sloughed mucosal cells and/or an increase in biliary cholesterol delivery into the lumen because of the elevated rate of hepatic cholesterol synthesis (Aqul et al., 2014). A comparison of the sterol excretion data for the Lal\(^{-/-}\) mice given PRD125 versus chow only (Fig. 4B) implies a marginal inhibition of cholesterol absorption in the treated mutants.

In contrast to the potency of PRD125 in blunting the rise in intestinal and hepatic EC levels, there was no discernible impact on the entrapment of TAG, at least in the liver. This was also true in Lal\(^{-/-}\);Soat2\(^{-/-}\) mice (Lopez et al., 2014). In that model and in Lal\(^{-/-}\) mice given PRD125, hepatic TAG concentrations (mg/g) were unchanged in the face of decisive falls in EC concentrations. Whole liver TAG contents (mg/organ) were reduced only marginally because of the decrease in liver mass. This result was not surprising given that a lowering of the EC content of nascent VLDL or chylomicrons because of the absence or suppression of SOAT2 activity should not cause a commensurate fall in the TAG content of these particles. In Lal\(^{-/-}\) mice given repeated injections of recombinant LAL, marked reductions in tissue cholesterol and TAG occurred (Sun et al., 2014). It is noteworthy that SOAT2 deficiency prevented cholesterol-associated steatosis in a mouse model by enhancing TAG mobilization (Alger et al., 2010). However, in that model, the excess TAG is contained in lipid droplets outside lysosomes. It is not known whether autophagocytosis of intracellular lipid droplets (Dong and Czaja, 2011) contributes to the buildup of EC and TAG in LAL deficiency, especially in the liver, or the extent to which such
A contribution might shift in the face of treatment with a SOAT2 inhibitor.

As is the case with most classes of cholesterol-lowering agents, inhibitors of cholesterol esterification are designed primarily to treat dyslipidemia. Here, an important point should be made about plasma cholesterol levels in LAL-deficient mice. While there are many similarities in mouse models for CESD and humans with this disorder, the type of dyslipidemia seen in human LAL deficiency (Reiner et al., 2014) is not replicated in \( \text{Lal}^{2/2} \) mice. Although published data are limited, it appears that in \( \text{Lal}^{2/2} \) mice, there is a shift in lipoprotein composition, but no change in the plasma total cholesterol concentration (Du et al., 1998, 2001). It remains to be determined whether this is the case in all strains of mice with LAL deficiency.

The data from both PRD125-treated \( \text{Lal}^{1/-} \) mice and \( \text{Lal}^{1/-}:\text{Soat2}^{1/-} \) mice indicate that while cholesterol esterification via SOAT2 is a major factor in driving disease progression in LAL deficiency, the role of SOAT1 in this process remains to be defined in more quantitative terms. It may be appreciable because SOAT1 is found in many cell types, in particular, macrophages, which have an increasing presence throughout the body as disease progresses (Du et al., 2001). Models that might yield clearer insights into the role of SOAT1 in CESD would be either \( \text{Lal}^{1/-}:\text{Soat1}^{1/-} \) mice or \( \text{Lal}^{1/-} \) mice treated with PRD125, together with moderate doses of the nonspecific SOAT inhibitor F1394 (Rong et al., 2013).

With respect to the therapeutic potential of PRD125 for managing CESD, it will now be necessary to investigate its efficacy in \( \text{Lal}^{1/-} \) mice that are at a more advanced stage of disease than is manifested in the 21-day-old animal. The data in Table 1 suggest that PRD125 treatment commencing at about 50 days of age and continuing for 3 months might establish whether sustained SOAT2 inhibition could serve as an adjunctive treatment option for enzyme replacement therapy.

Acknowledgments

The gift of \( \text{Lal} \) heterozygous mice, together with a genotyping protocol, from Drs. Gregory Grabowski and Hong Du is gratefully acknowledged. The authors also thank Dr. Joyce Repa for making the primers used for the quantitative polymerase chain reaction analyses available. Stephen Ostermann and Monti Schneiderman provided excellent assistance with animal care and stool collections.
Fig. 6. Indices of liver function in Lat<sup>+/+</sup> and Lat<sup>-/-</sup> mice given PRD125. Plasma activities of ALT and aspartate aminotransferase (AST) were determined in the same mice used in the experiment described in the legend to Fig. 2. Values are the mean ± S.E.M. of data from five animals in each group. Different letters denote statistically significant (P < 0.05) differences between values, as determined by two-way analysis of variance, with genotype and treatment as the variables.

Authorship Contributions

Participated in research design: Turley, Lopez, Posey, Rudel, Tomoda, Ohshiro.

Conducted experiments: Lopez, Chuang, Posey, Turley.

Contributed new reagents or analytic tools: Tomoda, Ohshiro.

Performed data analysis: Posey, Lopez, Chuang, Turley.

Wrote or contributed to the writing of the manuscript: Turley, Lopez, Chuang, Rudel, Tomoda, Ohshiro.

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