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Inhibition of ileal apical but not basolateral bile acid transport reduces atherosclerosis in apoE$^{-/-}$ mice

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ABSTRACT

Objective: Interruption of the enterohepatic circulation of bile acids induces hepatic bile acid synthesis, increases hepatic cholesterol demand, and increases clearance of apoB-containing lipoproteins in plasma. Based on these effects, bile acid sequestrants have been used for many years to treat hypercholesterolemia and the associated atherosclerosis. The objective of this study was to determine the effect of blocking ileal apical versus basolateral membrane bile acid transport on the development of hypercholesterolemia and atherosclerosis in mouse models.

Methods and results: ApoE$^{-/-}$ and Ldlr$^{-/-}$ mice deficient in the apical sodium-dependent bile acid transporter (Asbt) or apoE$^{-/-}$ mice deficient in the basolateral bile acid transporter (Ostα) were fed an atherogenic diet for 16 weeks. Bile acid metabolism, cholesterol metabolism, gene expression, and development of atherosclerosis were examined. Mice deficient in Asbt exhibited the classic response to interruption of the enterohepatic circulation of bile acids, including significant reductions in hepatic and plasma cholesterol levels, and reduced aortic cholesteryl ester content. Ileal Fibroblast Growth Factor-15 (FGF15) expression was significantly reduced in Asbt$^{-/-}$apoE$^{-/-}$ mice and was inversely correlated with expression of hepatic cholesterol 7-hydroxylase (Cyp7a1). Ileal FGF15 expression was directly correlated with plasma cholesterol levels and aortic cholesterol content. In contrast, plasma and hepatic cholesterol levels and atherosclerosis development were not reduced in apoE$^{-/-}$ mice deficient in Ostα.

Conclusions: Decreases in ileal FGF15, with subsequent increases in hepatic Cyp7a1 expression and bile acid synthesis appear to be necessary for the plasma cholesterol-lowering and atheroprotective effects associated with blocking intestinal bile acid absorption.

1. Introduction

Bile acids are synthesized in the liver, secreted into bile, and delivered to the small intestine, where they act to facilitate absorption of fats, cholesterol, and fat-soluble vitamins. Bile acids are almost quantitatively reclaimed from the intestine, and carried in the portal venous circulation back to the hepatocyte, where they are reabsorbed and resecreted into bile [1]. About 5% of the bile acids entering the small intestine escape reabsorption and are eliminated in the feces. Hepatic conversion of cholesterol to bile acid balances fecal excretion, and this process is the major route for cholesterol catabolism, accounting for almost half of the cholesterol eliminated from the body per day [2]. Disruption of the enterohepatic circulation (EHC) of bile acids stimulates de novo hepatic bile acid synthesis, thereby increasing hepatic cholesterol demand, cholesterol synthesis, and LDL receptor expression. This is the basis for the decreased plasma LDL cholesterol levels following ingestion of polymeric bile acid sequestrants [3,4], administration of ileal bile acid transporter inhibitors [5,6], or ileal bypass surgery such as in the POSCH study (Program on the Surgical Control of Hyperlipidemias) [7]. The transporters that function to maintain the EHC of bile acids have been identified [8]. In the ileum, the apical sodium-dependent bile acid transporter (Asbt or ibat; gene symbol Slc10a2) mediates the high capacity uptake of bile acids from the gut lumen across the brush border membrane of the enterocyte, whereas the heteromeric Organic Solute Transporter Ostα-Ostβ (gene symbols: Osta, Slc51a; Ostb, Slc51b) is responsible for basolateral membrane export of bile acids. Inactivation of either Asbt or Ostα impairs intestinal bile acid transport. However, Ostα null mice fail to induce hepatic expression of cholesterol 7α-hydroxylase (Cyp7a1) and bile acid synthesis [9,10], in striking contrast to a block in ileal apical brush border membrane uptake of bile acids [11,12]. This is thought to be due to an inability to down-regulate expression of the ileal-derived polypeptide hormone fibroblast growth factor (FGF) 15

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(human ortholog: FGF19) in Ostα$^{-/-}$ mice [13]. In addition to the flux of bile acids from intestine to liver, a critical role for gut-liver signaling via FGF15 has been recognized in the regulation of hepatic bile acid synthesis [14]. In this process, bile acids signal through the nuclear receptor FXR in ileal enterocytes to induce production of FGF15, which is secreted and carried to the liver, where it signals through the Fibroblast Growth Factor Receptor-4 (FGFR4)/β-klotho complex to repress Cyp7a1 expression and bile acid synthesis [15].

In light of the observed differences in bile acid metabolism between Asbt$^{-/-}$ and Ostα$^{-/-}$ mice, the aim of this study was to determine the effect of blocking ileal apical versus basolateral membrane bile acid transport on the development of hypercholesterolemia and atherosclerosis in mouse models. A second goal was to explore the relationship between ileal FGF15 expression and the atheroprotective effects associated with interruption of the EHC circulation of bile acids.

2. Materials and methods

For detailed methodology, please refer to the data supplement.

2.1. Mice and diets

The Institutional Animal Care and Use Committee approved all experiments. The Asbt$^{-/-}$ (129S6/SvEv) and Ostα$^{-/-}$ (C57BL/6) mice were generated as described previously [9,11] and crossbred with apoE$^{-/-}$ (C57BL/6) or Ldlr$^{-/-}$ (C57BL/6) to generate the Asbt$^{-/-}$apoE$^{-/-}$, Asbt$^{-/-}$Ldlr$^{-/-}$, and Ostα$^{-/-}$apoE$^{-/-}$ mice. At 6 weeks of age the mice were switched from rodent chow to an atherogenic diet for 16 weeks. The atherogenic diet contained 11% fat, 18% protein, 71% carbohydrate (% of Calories) and 0.280 mg/Calorie of cholesterol (0.1% w/w) [16].

2.2. Plasma, liver, biliary, and fecal lipid analysis

A fasting blood sample (4 h fast) was collected at the indicated time points to measure total plasma cholesterol (TPC) and triglyceride (TG). Plasma lipoprotein cholesterol distribution was quantified by FPLC size fractionation analysis. Hepatic levels of total cholesterol, free cholesterol, and TG were determined by enzymatic assay (Roche Applied Science) [11]. Gallbladder bile was used to measure phospholipid and bile acid by enzymatic assay; cholesterol was measured by gas–liquid chromatography. Individual bile acid species were quantified by HPLC. Feces were collected to measure total BA content by enzymatic assay and neutral sterol content by gas–liquid chromatography [11].

2.3. Quantification of aortic lipid content and lesion area

Whole aorta (from the sinotubular junction to iliac bifurcate) was removed, fixed in 10% formalin, and the adventitia was cleaned. For Ostα$^{-/-}$apoE$^{-/-}$ and matched apoE$^{-/-}$ mice, aortas were opened along the longitudinal axis and analyzed to quantify the percentage of total aortic surface covered with lesion. After surface lesion quantification, aortic total and free cholesterol concentrations were quantified by gas–liquid chromatography and normalized to aortic protein content. For Asbt$^{-/-}$apoE$^{-/-}$ and Asbt$^{-/-}$Ldlr$^{-/-}$ mice, aortic cholesterol content but not aortic lesion surface area was measured.

2.4. RNA and protein analyses

Total RNA was extracted from frozen tissue using TRIzol Reagent (Invitrogen). Real time PCR analysis was performed as described [13]. Tissue extracts were prepared and subjected to immunoblotting analysis using an affinity-purified rabbit anti-mouse FGF15 antibody [13]. Blots were also probed with anti-β-actin antibody. Protein expression was quantified by densitometry, and expression data were normalized to levels of the β-actin loading control.

2.5. Statistical analyses

Mean values ± SEM are shown unless otherwise indicated. The data were evaluated for statistically significant differences using the two-tailed Student's t test. Plasma cholesterol and TG levels were evaluated for statistically significant differences using a 2-way repeated measures ANOVA with genotype and time as factors and post hoc analyses using the Tukey–Kramer honestly significant difference test. The correlations were calculated using a Spearman Test. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Effect of Asbt deficiency on plasma lipids, hepatic lipids, and atherosclerosis

The Asbt$^{-/-}$apoE$^{-/-}$ and Asbt$^{-/-}$Ldlr$^{-/-}$ mice were indistinguishable from their apoE$^{-/-}$ and Ldlr$^{-/-}$ littermates in terms of survival, gross appearance, and behaviors. As previously noted for the Asbt$^{-/-}$ mice [17], there was a small but significant decrease in body weight and liver weight in Asbt$^{-/-}$apoE$^{-/-}$ versus and apoE$^{-/-}$ mice after 16 weeks on diet; a similar decrease in liver but not body weight was also observed in Asbt$^{-/-}$Ldlr$^{-/-}$ versus Ldlr$^{-/-}$ mice (Supplemental Table 2). Plasma lipids were measured at baseline and during the 16-week diet study. As analyzed by repeated measures ANOVA, there was a significant decrease in TPC in the Asbt$^{-/-}$apoE$^{-/-}$ mice versus apoE$^{-/-}$ mice over the 16-week atherogenic diet challenge (Fig. 1A). There was also statistically significant reduction in the calculated TPC area under the curve (AUC) as evaluated using the two-tailed Student's t test (18,067 + 1448 versus 23,222 + 1898 Arbitrary units; $n = 13–16$, $p < 0.05$). Asbt$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice also had higher plasma TG levels (Fig. 1B). After 16 weeks on diet, plasma was collected and lipoproteins were fractionated by FPLC to determine the cholesterol distribution. The cholesterol concentration was significantly lower for the apoB-containing lipoprotein fraction (VLDL and LDL) but not for the HDL fraction in Asbt$^{-/-}$apoE$^{-/-}$ mice (Fig. 1C and D). Hepatic lipids were measured after 16 weeks on diet; cholesteryl ester content was decreased by 68% in Asbt$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice, but hepatic TG content remained elevated in both genotypes (302 + 21 versus 347 + 26 μg TG/mg liver wet weight in Asbt$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice) (Fig. 1E). Aortic total cholesterol (Fig. 1F) and cholesteryl ester (2.66 + 0.77 versus 16.57 + 2.52 μg/mg protein; $n = 13–16$, $p < 0.05$) content was significantly reduced by approximately 54% and 84%, respectively, in Asbt$^{-/-}$apoE$^{-/-}$ versus apoE$^{-/-}$ mice. As shown in Supplemental Fig. 1, reductions were also observed for TPC, apoB-containing lipoprotein (VLDL plus LDL) cholesterol, hepatic cholesteryl ester content, and aortic total cholesterol and cholesteryl ester content (5.49 + 1.01 versus 9.08 + 1.26 μg/mg protein; $n = 15–19$, $p < 0.05$) in Asbt$^{-/-}$Ldlr$^{-/-}$ versus Ldlr$^{-/-}$ mice. In addition, hepatic TG levels were also reduced in Asbt$^{-/-}$Ldlr$^{-/-}$ versus Ldlr$^{-/-}$ mice (Supplemental Fig. 1E). These findings are in general agreement with previous reports of apoE$^{-/-}$ and Ldlr$^{-/-}$ mice treated with a bile acid sequestrant [18,19], and apoE$^{-/-}$, Ldlr$^{-/-}$/apoE$^{-/-}$, and SR-BI$^{-/-}$/apoE$^{-/-}$ mice treated with Asbt inhibitors [20–22]. As the findings were similar in the Asbt$^{-/-}$apoE$^{-/-}$ and Asbt$^{-/-}$Ldlr$^{-/-}$ mice, subsequent studies focused on examining the effects of Ostα deficiency in the apoE$^{-/-}$ mice.