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SCD1 deficiency protects mice against ethanol-induced liver injury

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ABSTRACT

Stearoyl-CoA desaturase 1 (SCD1) is a delta-9 fatty acid desaturase that catalyzes the synthesis of mono-unsaturated fatty acids (MUFA). SCD1 is a critical control point regulating hepatic lipid synthesis and β-oxidation. *Scd1* KO mice are resistant to the development of diet-induced non-alcoholic fatty liver disease (NAFLD). Using a chronic-binge protocol of ethanol-mediated liver injury, we aimed to determine if these KO mice are also resistant to the development of alcoholic fatty liver disease (AFLD).

Mice fed a low-fat diet (especially low in MUFA) containing 5% ethanol for 10 days, followed by a single ethanol (5 g/kg) gavage, developed severe liver injury manifesting as hepatic steatosis. This was associated with an increase in *de novo* lipogenesis and inflammation. Using this model, we show that *Scd1* KO mice are resistant to the development of AFLD. *Scd1* KO mice do not show accumulation of hepatic triglycerides, activation of *de novo* lipogenesis nor elevation of cytokines or other pro-inflammatory markers. Incubating HepG2 cells with a SCD1 inhibitor induced a similar resistance to the effect of ethanol, confirming a role for SCD1 activity in mediating ethanol-induced hepatic injury.

Taken together, our study shows that SCD1 is a key player in the development of AFLD and associated deleterious effects, and suggests SCD1 inhibition as a therapeutic option for the treatment of this hepatic disease.

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1. Introduction

Alcohol consumption and abuse is a risk factor of chronic disease worldwide and has long been identified as a major risk factor for all liver diseases [1]. Liver diseases induced by ethanol abuse range from simple fatty liver to more severe forms of liver injury such as alcoholic hepatitis, cirrhosis and hepatocellular carcinoma [2,3]. A fatty liver, also known as alcoholic fatty liver disease (AFLD), is the earliest sign of ethanol-induced liver injury. This fat accumulation (*i.e.*, steatosis) is usually accompanied by inflammation [4]. AFLD occurs in 80% of heavy drinkers who consume an excess of 80 g ethanol per day [5]. This state is usually asymptomatic and can be reversed after 4 to 6 weeks of abstinence [6]. However, continued heavy ethanol consumption increases by 30% the risk of progression to cirrhosis [7]. Unfortunately, 5–15% of patients with AFLD develop fibrosis and cirrhosis despite abstinence [8].

Approximately 90% of ingested ethanol is metabolized in the liver [4]. Ethanol is first oxidized to acetaldehyde by alcohol dehydrogenase and partly metabolized by cytochrome P-450 and catalase in hepatocyte microsomes and perixosomes, respectively [4]. Acetaldehyde is the major toxin in ethanol-induced liver injury, causing cellular damage, inflammation and fibrosis [9]. Acetaldehyde also increases the redox ratio of nicotinamide adenine dinucleotide ($NADH/NAD^+$) leading to a reduction of fatty acid β-oxidation through a reduction of PPARα activity [4,10]. Activation of hepatic lipogenesis is another important biochemical characteristic of hepatic steatosis in AFLD development [11]. You and collaborators have shown that ethanol induces fatty acid synthesis by acetaldehyde-mediated activation of the sterol regulatory element-binding protein (SREBP)-1 (12). Notably, SREBP-1 plays a central role in lipid metabolism by regulating the transcription of genes involved in hepatic lipid synthesis such as *SREBP-1* itself, *ACC*, *FAS*, and *SCD1* [13]. Acetaldehyde is then converted to acetate by acetaldehyde dehydrogenase (ALDH) in mitochondria of hepatocytes [9]. High hepatic levels of acetate, the main substrate of ACC, can lead to the formation of acetyl-CoA in hepatocytes [14]. In addition to lipid accumulation, the production of TNF-α is one of the earliest responses to ethanol-induced liver injury [15]. TNF-α, a mediator of the mammalian inflammatory response, up-regulates hepatic *SREBP-1* mRNA expression, activates its maturation and reduces PPARα expression (4). Taken together, all these modifications lead to the aggravation of liver injury [4].

SCD1 is a delta-9 fatty acid desaturase that catalyzes the synthesis of 16:1(n−7) and 18:1(n−9) mono-unsaturated fatty acids (MUFA). SCD1 is a key enzyme in the regulation of hepatic lipogenesis and β-oxidation of lipids [16]. Increases in the hepatic desaturation index resulting from elevated SCD1 expression and activity have been

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associated with non-alcoholic fatty liver (NAFLD) and metabolic syndrome [17]. Several studies have shown that *Scd1*-deficient mice are protected against obesity and NAFLD [18,19]. *Scd1* KO mice show reduced lipid synthesis and enhanced β-oxidation of lipids, as well as increased thermogenesis and insulin sensitivity in various tissues, including the liver. These metabolic changes protect *Scd1* KO mice from a variety of dietary, pharmacological, and genetic conditions that promote hepatic steatosis.

Ethanol induces an increase in *de novo* lipogenesis *via* increased expression of key genes implicated in lipid metabolism [12]. Due to its central role in hepatic lipid metabolism and its implication in the development of NAFLD [4], we evaluated the role of *Scd1* in the development of AFLD. We show that *Scd1* KO mice are completely protected against AFLD, suggesting that decreasing SCD1 activity can offer a new therapeutic strategy for the treatment of this disease.

2. Materials and methods

2.1. Mice

Wild-type (WT) C57BL/6N mice were purchased from Charles River laboratories (Senneville, Canada). *Scd1* knockout (*Scd1* KO) mice in the C57BL/6N background originated from the Ntambi laboratory (University of Wisconsin-Madison, Madison, USA) and were generated in the University of Quebec in Montreal (UQAM) animal facilities. Only male mice were used in the study. The UQAM Animal Care and Use Committee approved all animal experimental protocols.

2.2. Chronic + binge protocol of alcoholic fatty liver disease induction

Twelve week-old male WT and *Scd1* KO mice were acclimated to a low-fat liquid control diet (AIN-76 (F1268); Bio-Serv, Frenchtown, USA) for 5 days. Thereafter, the animals were separated in four groups and divided into 2 or 3 mice per cage: WT with control diet (CTRL) (n = 8), *Scd1* KO with control diet (*Scd1* KO) (n = 5), WT with ethanol diet (EtOH) (n = 8) and *Scd1* KO with ethanol diet (*Scd1* KO EtOH) (n = 8). The ethanol groups (EtOH and *Scd1* KO EtOH) were fed a liquid diet (AIN-76 (F1436); Bio-Serv, Frenchtown, USA) containing 5% ethanol for 10 days while the CTRL group was maintained on the control diet. The caloric breakdown and fatty acid composition of these diets is described in Table 1. At day 11, mice in EtOH groups were gavaged a single dose of EtOH (5 g/kg body weight, 31.5% solution), whereas mice in the control group were gavaged an isocaloric dose of dextrin maltose (45% solution). The gavage was always performed in the early morning. After gavage, mice were kept on control or EtOH diet for an additional 9 h. During this time, the animals were kept on a warm electric blanket. After gavage, mice were slow moving, but conscious and regained normal behavior within 4–6 h. The mice were euthanized 9 h after gavage, when ALT and AST serum levels reach their peak [20].

Table 1
Caloric breakdown and fatty acid composition of liquid diets.

	Control liquid diet		Ethanol liquid diet	
	AIN-76 (Bio-Serv #F1268)		AIN-76 (Bio-Serv #F1436)	
Caloric breakdown				
	kcal/L	% kcal	kcal/L	% kcal
Protein	180	18.0	173	17.3
Fat	125	12.5	124	12.4
Carbohydrate	*695*	*69.5*	*348*	*34.8*
Ethanol	–	–	*355*	*35.5*
Total	1000	100	1000	100
Fatty acid composition				
	g/L	g/100 g total fat	g/L	g/100 g total fat
Saturated	1.7	13.3	1.7	13.3
Monounsaturated	3.7	28.9	3.7	28.9
Polyunsaturated	7.4	57.8	7.4	57.8
Total	12.8	100	12.8	100

Categories in italics add up to similar kcal levels in control versus ethanol diets.

2.3. Plasma transaminase assays

Blood was collected from mice by cardiac exsanguination in collection tubes containing 10 IU/mL Heparin. The blood was then centrifuged for 10 min at 200*g* and the plasma was collected for subsequent analysis. Aspartate transaminase (AST) and alanine transaminase (ALT) activities were measured using a Beckman DxC automated Monarch device (Biochemical laboratory, Notre-Dame Hospital, Montréal, Canada).

2.4. Liver histology

To detect fat deposition in the liver, frozen samples of livers were embedded in Optimal Cutting Temperature compound (Fisher Scientific, Hampton, USA), sectioned (4–8 μm) using a cryostat (Leica Biosystems, Wetzlar, Germany) and fixed for 10 min in 4% paraformaldehyde. Then sections were stained with Oil Red-O (Sigma-Aldrich, Saint Louis, USA) for lipid accumulation and nuclei were lightly stained with Hematoxylin. Hematoxylin and Eosin (H&E) staining was used to evaluate neutrophil infiltration [21]. Images were acquired with an A1 Nikon microscope using a 20× objective lens and a color camera.

2.5. Immunofluorescence

Immunofluorescence was used to evaluate the expression of F4/80, a macrophage marker. We used 8 μm sections of livers from different groups as described above. Sections were then permeabilized for 10 min using PBS 1× containing 0.25% Triton-×100 (PBST), blocked for 30 min in 1% bovine serum albumin in PBST and finally incubated with Alexa Fluor 647 F4/80 antibody (1:250) (BioLegend, London, UK). Nuclei were then stained with DAPI and images were acquired with an A1 Nikon confocal microscope using a 20× objective lens.

2.6. Triglyceride assay

Concentration of liver triglycerides was determined using a colorimetric assay kit from Cayman Chemical (Ann Arbor, USA). Briefly, 200 mg of liver tissue was homogenized in 1 mL of Standard Diluent containing complete protease inhibitor cocktail (Sigma-Aldrich, Saint Louis, USA). The homogenate was then centrifuged at 4 °C for 10 min at 10,000*g*. The supernatant was collected and hepatic triglycerides were measured by enzymatic hydrolysis of triglycerides to glycerol and free fatty acids, followed by colorimetric measurement (at 540 nm wavelength) of glycerol. Values for hepatic triglycerides were expressed as mg of triglyceride per g of liver tissue.

2.7. HepG2 cell culture

HepG2, a human hepatoma cell line, was obtained from ATCC (Manassas, USA) and maintained in Eagles minimum essential medium (EMEM; Wisent, St-Jean-Baptiste, Canada) supplemented with 10% fetal bovine serum (Life Technologies, Grand Island, USA). Twenty-four hours before treatments, cells were starved in serum free medium. Ethanol-induced injury was achieved by exposing cells to EtOH (50 mM) in serum free medium for a total of 48 h, the last 24 h of which where spent in the presence of 1 μM SCD1 inhibitor A939572 (Biofine, Vancouver, Canada) where appropriate.

2.8. Lipid droplet imaging

Control and ethanol-treated HepG2 cells were washed three times with ice-cold 1× PBS and fixed in 4% paraformaldehyde for 30 min. Lipid droplets were stained for 10 min with 1 μg/mL Bodipy 493/503