Brief communication

MOLECULAR METABOLISM

The LXR inverse agonist SR9238 suppresses fibrosis in a model of non-alcoholic steatohepatitis

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ABSTRACT

Objective: Non-alcoholic steatohepatitis (NASH) is characterized by hepatic steatosis, inflammation and fibrosis. There are currently no targeted therapies for NASH. We developed a liver-specific LXR inverse agonist, SR9238, which effectively reduces hepatic lipogenesis in models of obesity and hepatic steatosis. We hypothesized that suppression of lipogenesis, which is pathologically elevated in NASH may suppress progression of hepatic steatosis to NASH.

Methods: NASH was induced in B6 V-lep ^{ob}/J (*ob*/*ob*) mice using a custom complete rodent diet (HTF) containing high amounts of trans-fat, fructose, and cholesterol. Once NASH was induced, mice were treated with SR9238 for one month by i.p. injection. Plasma lipid levels and liver health were analyzed by clinical chemistry. QPCR, western blot, and immunohistochemistry were used to assess disease severity.

Results: *Ob*/ob mice are obese and diabetic thus they are commonly used as models for the study of metabolic diseases. These mice quickly developed the NASH phenotype when provided the HTF diet. The mice develop hepatic steatosis, severe hepatic inflammation and fibrosis on the HTF diet. Treatment with SR9238 significantly reduced the severity of hepatic steatosis and most importantly reduced hepatic inflammation and ameliorated hepatic fibrosis.

Conclusions: Here, we demonstrate that an LXR inverse agonist, SR9238, is effective in reduction of hepatic steatosis, inflammation and fibrosis in an animal model of NASH. These results have important implications for the development of therapeutics for treatment NASH in humans.

Keywords NASH; Fibrosis; Lipogenesis; LXR; Inflammation

1. INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is a severe form of chronic liver disease, characterized by steatosis, inflammation, and pericellular fibrosis [1—4]. It is often associated with obesity, dyslipidemia, insulin resistance, and hyperglycemia, and can progress to cirrhosis or hepatocellular carcinoma. Current therapies often target the associated metabolic disease but not NASH directly [5]. Development of non-alcoholic fatty liver disease (NAFLD) is the first step towards NASH and is characterized by hepatic steatosis that develops due to an imbalance of triglyceride removal and acquisition within the liver and is also associated with elevated hepatic lipogenesis [2,4]. High calorie diets with excessive fats and carbohydrates can lead to this imbalance leading to NAFLD and in some cases, progression to NASH.

The Liver X Receptors (LXRα and LXRβ) are members of the nuclear receptor (NR) superfamily and are widely expressed (LXRα is primarily expressed in the liver, kidneys, intestines, and adipose tissues while LXRβ is ubiquitously expressed) [6,7]. LXRs have been shown to regulate cholesterol efflux and transport, as well as stimulate hepatic lipogenesis [6,8,9]. Synthetic LXR agonists have been shown to display anti-atherogenic properties due to their effects on reverse cholesterol transport mediated by increased cholesterol efflux from peripheral tissues [1,6,8,10,11]. However, the activation of LXR by synthetic ligands results in deleterious effects due to increased hepatic lipogenesis due to increased expression of lipogenic enzymes including fatty acid synthase (*Fasn*) and sterol regulatory element-binding protein (*Srebp1*) that are direct target genes of LXR. This has caused significant difficulties in the development of LXR agonists as anti-atherogenic therapeutics [8,10—12].

We believed that regulation of lipogenesis by LXR might be an opportunity to focus on diseases that are characterized by elevated lipogenesis such as NAFLD and NASH. To this end, we developed LXR inverse agonists that have the ability to suppress the expression of LXR target genes such as *Fasn* and *Srepb1* [13]. Previously, we demonstrated that the LXR inverse agonist, SR9238, which displays liver selectivity, is effective in suppressing hepatic steatosis associated with

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Abbreviations: NASH, non-alcoholic steatohepatitis; LXR, Liver-X-Receptor; HTF, high trans-fat diet; NR, nuclear receptor

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a high fat diet in mice [13]. In this study, we sought to determine if such a therapeutic would have efficacy in reduction of inflammation and fibrosis in a mouse model of NASH.

2. METHODS

2.1. Animals

Six-week-old B6 V-Lep^{ob}/J (*ob*/*ob*) mice were purchased from Jackson Laboratories (Bar Harbor, ME), housed individually in standard cages, and immediately placed on D09100301 (NASH) diet (Research Diets). This is a High Trans-Fat (Primex shortening-based) diet that also contains 20% kcal from fructose and 2% cholesterol. Mice were maintained on the diet for six weeks prior to starting treatment, handled daily for acclimation and weighed every other day during this time. Groups were weight-matched prior to beginning treatment ($n = 7$). After six weeks, mice continued on the NASH diet and were treated with 30 mg/kg SR9238 q.d. i.p. in 10%DMSO/10%Tween-80/80%water or vehicle for 30 days. Body weight and food intake were monitored daily, blood glucose was determined weekly using a hand-held glucometer, and a final fasting blood glucose was collected at termination. At the termination of dosing, blood was collected by cardiac puncture and analyzed using clinical chemistry (Roche cobas c311) and ELISA. Liver was collected, weighed, and a portion was immediately flash frozen in liquid nitrogen for RNA. The rest was placed in 10% neutral buffered formalin for histology. The Institutional Animal Care and Use Committee at the Scripps Research Institute, Jupiter FL, approved all mouse studies.

2.2. Gene and protein expression studies

RNA was isolated from liver and analyzed by QPCR as previously described in Ref. [9]. Each sample was run in duplicate and measured using the ddCT method, using *Gapdh* as the reference gene. Liver protein lysate was isolated by standard methods in RIPA buffer containing Roche miniComplete protease inhibitor. Total protein was quantified by a 96-well format BCA assay (ThermoFisher) and diluted in 2× Laemmli Buffer (Amresco) for western blot analysis. Liver lysates (25 μg) were separated on miniTGX anyKd gels (Bio-Rad) and transferred to nitrocellulose membranes. Membranes were blocked in 5% Non-fat Milk in TBST for 1 h, then probed either with FASn (1:1000; Cell Signaling), TGFβ (1:1000; Abcam), SREBP2 (1:750; Abcam) or Actin (1:1000; Cell Signaling). Following incubation with HRP anti-rabbit antibody (1:10,000; Santa Cruz Biotechnology),

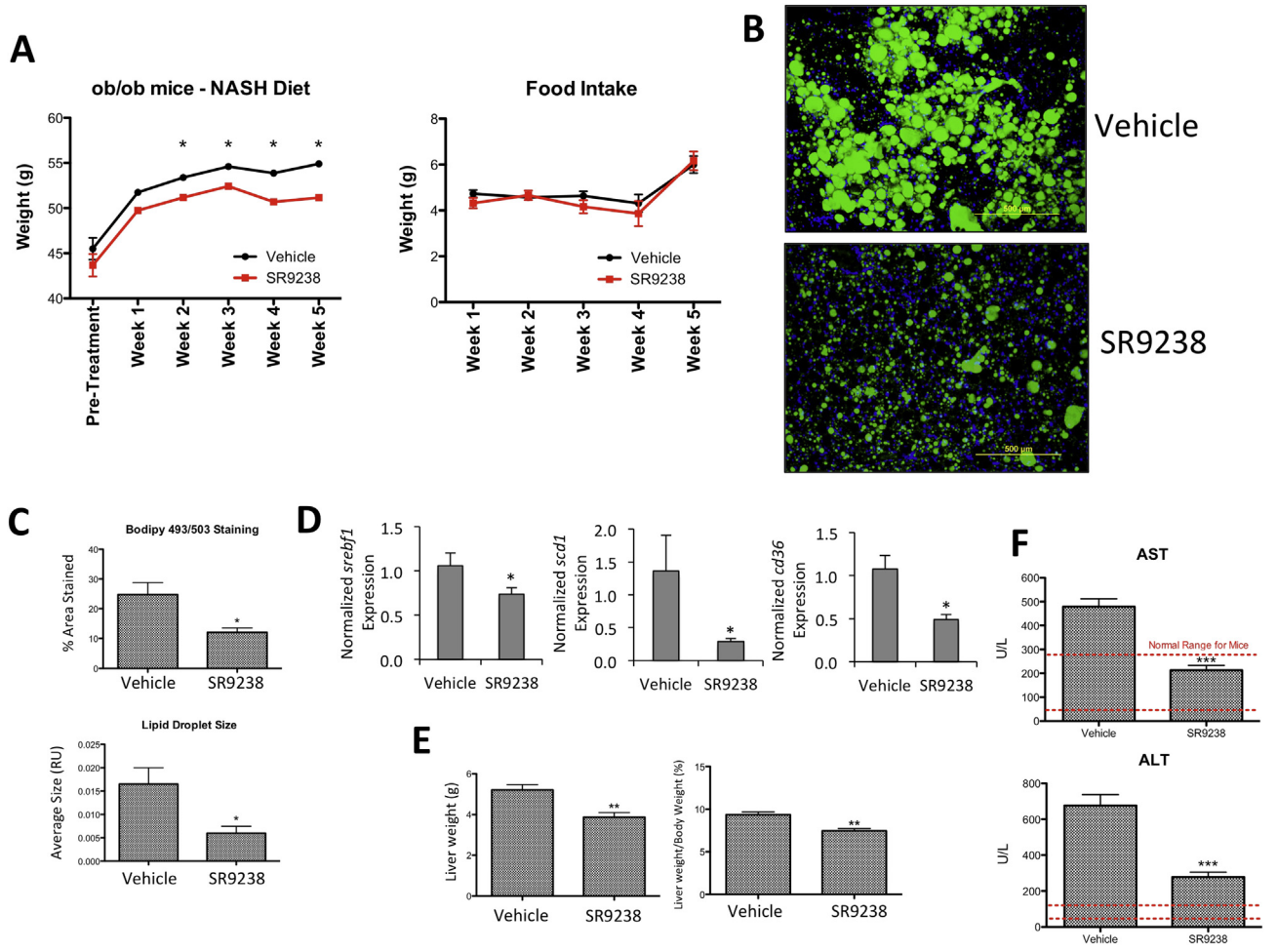

Figure 1: SR9238 significantly reduces hepatic lipids and normalizes liver function in NASH mice. (A) Body weight of mice on NASH diet treated with SR9238 or vehicle (left panel). Averaged daily food intake of mice during treatment period (right panel) (B) Bodipy 493/503 lipid staining of liver sections. (C) Quantitation of bodipy staining by % Area Stained and Lipid Droplet size using ImageJ software (NIH). (D) Hepatic *Srepf1*, *scd1* and *cd36* gene expression is suppressed by SR9238 treatment. Gene expression was determined by QPCR and normalized to *gapdh* expression. (E) SR9238 treatment reduces liver and total body weight. (F) Reduced liver enzyme levels suggest that SR9238 treatment significantly decreases hepatocellular damage. *indicates $p < 0.05$.

