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Farnesoid X receptor agonist WAY-362450 attenuates liver inflammation and fibrosis in murine model of non-alcoholic steatohepatitis☆

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***Background/Aims*: The farnesoid X receptor (FXR) is a member of the nuclear hormone receptor superfamily, which plays an essential role in the regulation of enterohepatic circulation and lipid homeostasis. Here we investigated whether WAY-362450, a synthetic potent FXR agonist, could protect against non-alcoholic steatohepatitis (NASH) in mice fed a methionine and choline-deficient (MCD) diet.**

***Methods*: Male C57BL/6 mice on the MCD diet were treated with or without WAY-362450 (30 mg/kg) for 4 weeks.**

***Results*: The elevations of serum ALT and AST activities induced by the MCD diet were decreased with WAY-362450 treatment. In terms of liver histology, while WAY-362450 treatment showed no impact on hepatic triglyceride accumulation, it significantly reduced inflammatory cell infiltration and hepatic fibrosis. The reduction in inflammatory cell infiltration correlated with deceased serum levels of keratinocyte derived chemokine (mKC) and MCP 1 and decreased hepatic gene expression of MCP-1 and VCAM-1. The reduction of hepatic fibrosis by WAY-362450 treatment corresponded to a reduction in hepatic gene expression of fibrosis markers. The positive effects of WAY-362450 were FXR-dependent since no protection was observed in MCD diet-fed FXR deficient mice.**

***Conclusions*: These findings demonstrate that FXR agonists may be useful for the treatment of non-alcoholic steatohepatitis.**

***Keywords*: Farnesoid X receptor (FXR) agonist; Non-alcoholic steatohepatitis (NASH); WAY-362450; Methionine-and choline-deficient (MCD) diet**

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) refers to a spectrum of liver disorders ranging from hepatic steatosis to steatohepatitis and fibrosis [1–5]. Simple NAFLD, hepatic steatosis, is generally considered benign, whereas non-alcoholic steatohepatitis (NASH) is a potentially serious condition with poor prognosis.

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Abbreviations: FXR, farnesoid X receptor; SHP, small heterodimer partner; BSEP, bile salt export pump; MCD, methionine- and choline-deficient; NASH, nonalcholic steatohepatitis; NAFLD, non-alcoholic fatty liver disease; CYP7A1, cholesterol 7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; 6ECDCA, 6α-ethyl-chenodeoxycholic acid; Apo, apolipoprotein; $FXR^{-/-}$, FXR deficient; ALT, alanine aminotransferase; AST, aspartate aminotransferase; MCP-1, monocyte chemotactic protein-1; mKC, keratinocyte-derived chemokine; VCAM-1, vascular cell adhesion molecule-1; TIMP-1, tissue inhibitor of metalloproteinase 1; MMP-2, matrix metalloproteinase 2; PDK4, pyruvate dehydrogenase kinase; HSC, hepatic stellate cell.

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NASH patients are at great risk to progress to cirrhosis and liver failure, or to hepatocellular carcinoma [2–5]. NAFLD and NASH are frequently associated with metabolic syndrome including insulin resistance, diabetes, obesity, and hyperlipidemia [6–8]. Currently, there is no proven effective therapy for NASH, while liver transplantation is the only option for end-stage NASH cirrhosis [8]. Feeding mice a MCD diet is a well-established nutritional model of NASH with serum AST and ALT elevations, and liver histological changes similar to human NASH, including hepatic steatosis, lobular inflammation and pericelluar fibrosis [4,9,10].

The farnesoid X receptor (FXR; NR1H4) is a member of the nuclear hormone receptor superfamily that functions as ligand-activated transcription factors, and is highly expressed in the liver, intestine, kidney and adrenal glands [11]. FXR can be activated by physiological concentrations of bile acids [12–14], or by potent synthetic FXR ligands including GW4064, 6α-ethyl-chenodeoxycholic acid (6ECDCA) and WAY-362450 [15,16,40]. FXR regulates genes involved in bile acid synthesis, lipid and lipoprotein metabolism, including small heterodimer partner (SHP), cholesterol 7α-hydroxylase (CYP7A1), sterol 12α-hydroxylase (CYP8B1), bile salt export pump (BSEP), apolipoprotein (apo) AI, apo CII, apo CIII and the phospholipids transfer protein (PLTP) [17–22]. On the chow diet, hepatic triglyceride content of FXR deficient ($FXR^{-/-}$) mice was significantly higher (2.2-fold) than that of wild-type mice [18]. At ages 9–12 months, $FXR^{-/-}$ mice livers exhibited significant liver inflammation and injury, and spontaneously developed hepatocellular adenoma and carcinoma, which is similar to the end-stage of NASH [23,24]. Furthermore, FXR agonist has been shown to protect against cholestatic liver injury and fibrosis in rat models of extrahepatic and intrahepatic cholestasis [25], and attenuate liver fibrosis through inhibition of HSC activation [26,27].

Here we demonstrate that WAY-362450, a synthetic potent FXR agonist [40], protects against hepatic inflammation and fibrosis without inhibiting hepatic triglyceride accumulation in mice fed a methionine- and choline-deficient (MCD) diet. This hepatoprotection by WAY-362450 is totally abolished in $FXR^{-/-}$ mice.

2. Materials and methods

2.1. Animal studies

All procedures involving animals were reviewed and approved by the Wyeth Institutional Animal Care and Use Committee. C57BL/6 mice and $FXR^{-/-}$ mice on a C57BL/6 background (#004144) were obtained from Jackson Laboratories. Male 8- to 10-week-old C57BL/6 mice and $FXR^{-/-}$ mice were housed in cages and maintained under 12-h light-dark cycles with free access to food and water. Male C57BL/6 mice were divided into 3 experimental groups, fed and treated as follows for 4 weeks: (1) Control chow diet (Harlan Teklad, TD94149), (2) MCD diet (Harlan Teklad, TD90262), orally administered with vehicle (0.1 ml, corn oil/ethanol, 9/1) once daily for 4 weeks, or (3) MCD diet, orally administered with 30 mg/kg WAY-362450 in vehicle once daily for 4 weeks. We monitored food intake and found no difference between MCD/vehicle group and MCD/WAY362450 group. Male $FXR^{-/-}$ mice were tested in the same way as C57BL/6 mice. On the last day, mice were orally administered with vehicle or compounds in the morning, fasted for 3.5 h, then blood was collected by cardiac puncture, and livers were dissected and fixed in 10% formalin for histological analysis or snap frozen in lipid nitrogen for future analyses. Liver total RNA was prepared by using Trizol reagent (GIBCOBRL) and further purified using RNeasy kit (Qiagen). Gene expression analysis was performed with quantitative RT-PCR as described previously [28]. Sequences of gene-specific primer and probe sets designed with Primer Express Software (Applied Biosystems) were published previously for murine FXR, SHP and VCAM-1 [28]. Murine BSEP, MCP-1, TIMP-1, α1(I) collagen, α-SMA, TGF-β1, MMP-2 and α2(I) collagen probe/primer sets are in Supplemental Table. All results were normalized to GAPDH (4308313; PE Applied Biosystems, Foster City, CA) and are means ± SEM. Statistical significance was determined by ANOVA.

2.2. Morphological and biochemical analyses

Histology was performed as previously described with frozen sections (5 μm) using Oil Red O, hematoxylin and eosin (H&E), Masson's trichrome (Sigma) or Sirius Red staining [26,27,29]. H&E stained sections (3 randomly selected fields/section at ×20 magnification) were viewed blindly and scored for inflammation with 0 = no inflammatory foci, 1 = 1–2 inflammatory foci, 2 = 3–4 inflammatory foci, 3 = 4 and more inflammatory foci. Sirius red stained sections (3 randomly selected fields/section at ×20 magnification) were quantified by MetaVue imaging software (Molecular Devices, Downingtown, PA). Serum ALT (Roche kit# 12217317 001), and AST (Roche kit# 12217309 001) levels were quantified with a Roche/Hitachi 912 clinical chemistry analyzer (Roche Diagnostics, Indianapolis, IN). Serum MCP-1 (K152AYC-1) and mKC (K152BKC-1) levels were measured with commercial kits from Meso scale. Hepatic triglyceride content was determined as described previously [30].

3. Results

FXR-deficient mice exhibit a hepatic phenotype similar to NASH patients with significant hepatic triglyceride accumulation, hepatic inflammation and injury, and spontaneous hepatocellular carcinoma development [16,21–24]. Therefore, we examined whether the synthetic FXR agonist WAY-362450 could protect against the development of NASH in a murine MCD model. Adult male C57BL/6 mice were fed with MCD diet while being treated with either vehicle or WAY-362450 (30 mg/kg/day) for 4 weeks. At the end of the experiment, liver samples and serum were collected for further analyses.

3.1. Effects of WAY-362450 on serum liver functional enzymes, MCP-1 and mKC

A diagnostic marker for the presence of NASH has been the elevation in the serum liver functional enzymes, AST and ALT. As expected, serum activities of AST and ALT were both substantially elevated by MCD diet feeding (Fig. 1A) and WAY-362450 treatment resulted in significant reductions in these serum activities. Analyses of additional inflammatory serum markers demonstrated a