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Retinol dehydrogenase 13 deficiency diminishes carbon tetrachloride-induced liver fibrosis in mice

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HIGHLIGHTS

- Rdh13 deficiency reduces liver injury and fibrosis by attenuating hepatic stellate cell activation.
- Rdh13 deficiency reduces collagen I(II) and TIMP-1 expression during liver fibrosis.
- Rdh13 deficiency reduces TGF-β1 expression during liver fibrosis.

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ABSTRACT

Retinol dehydrogenase 13 (RDH13) is a mitochondrion-localized member of the short-chain dehydrogenases/reductases (SDRs) superfamily that participates in metabolism of some compounds. *Rdh13* mRNA is most highly expressed in mouse liver. Rdh13 deficiency reduces the extent of liver injury and fibrosis, reduces hepatic stellate cell (HSC) activation, attenuates collagen I (II), tissue inhibitor of metalloproteinase 1 (TIMP-1) and transforming growth factor beta 1 (Tgf-β1) expression. The results indicate an important role of Rdh13 and suggest RDH13 as a possible new therapeutic target for CCl_4-induced fibrosis.

1. Introduction

Retinol dehydrogenase 13 (RDH13) is a recently identified member of the short-chain dehydrogenases/reductases (SDRs) superfamily that participate in metabolism of prostaglandins, retinoids, steroids and aliphatic alcohols (Haeseleer et al., 2002; Levi et al., 2012; Oppermann et al., 2003). RDH13 shares sequence similarity with RDH11, RDH12 and RDH14 proteins which have been characterized as microsomal proteins that recognize retinoids and medium-chain aldehydes as substrates, with NADP+/NADPH as the preferred cofactors (Belyaeva et al., 2005). It has been demonstrated that RDH13 is localized on the outer side of the inner mitochondrial membrane and exhibits a wide tissue distribution. Purified RDH13 is catalytically active and recognizes retinoids as substrates, strongly prefers NADPH over NADH as a cofactor, and has a much greater catalytic efficiency as a reductase than as a dehydrogenase (Belyaeva et al., 2008). Our previous work show that Rdh13 plays an important role in retinal light damage which is consistent with Rdh11 and Rdh12 in mice (Wang et al., 2012). Thus, RDH13 is also considered to participate in the retinoid metabolism.

The liver is the most important organ in the body involved in retinoid storage and metabolism, and also an important target organ for retinoid actions. Within the liver, both hepatocytes and HSCs are importantly involved in retinoid metabolism (Shirakami et al., 2012). Retinoic acid is synthesized in the liver and can interact with retinoid receptors which control the expression of a

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large number of genes involved in hepatic processes. Altered retinoid metabolism and the accompanying dysregulation of retinoid signaling in the liver contribute to hepatic disease including fatty liver, liver fibrosis, cirrhosis and hepatocellular carcinoma (Shirakami et al., 2012). This is related to HSCs, which store more than 50% of all retinoid present in the body and contribute significantly to the development of hepatic disease when they undergo a process of cellular activation. HSC activation results in the loss of HSC retinoid stores and changes in extracellular matrix deposition leading to the onset of liver fibrosis. Within the liver, like in other tissues, retinol is converted to retinoic acid via two oxidative steps. Retinol dehydrogenases (RDHs) catalyze the first enzymatic step which is the oxidation of retinol to retinaldehyde. Several RDHs including RDH5, RDH10, RDH11, RDH1 and RDH13 are expressed in the liver and exhibit RDH activity in vitro (Shirakami et al., 2012). But the physiological role of each of them in retinoic acid synthesis and liver disease remains unclear. Our previous work showed that *Rdh13* mRNA is most highly expressed in mouse liver, which is 3–10 times that of other tissues. This suggests possible importance of Rdh13 in the retinoid signaling and hepatic pathology. Recently, we investigated the function of Rdh13 in liver and acute liver injury by using Rdh13-deficient mice. We found that there was no obvious difference in the serum biochemistry and the liver histology between wild-type and Rdh13-deficient mice under normal condition, and Rdh13-deficient mice showed reduced liver injury and hepatocyte proliferation compared to wild-type mice following carbon tetrachloride (CCl_4 administration.

Here, we use *Rdh13* knockout (KO) mice to investigate the role of Rdh13 in liver fibrosis after repeated CCl_4 administration. The results indicate that Rdh13 deficiency reduces the extent of liver injury and liver fibrosis, reduces HSC activation, attenuates collagen I(II), and TIMP-1 expression after chronic CCl_4 treatment. Our findings indicate an important role of Rdh13 in liver disease *in vivo* and suggest RDH13 as a possible new therapeutic target for the prevention and treatment of hepatic fibrosis in chronic liver disease.

2. Materials and methods

2.1. Animals

WT and *Rdh13* KO mice (8–10-week-old) with a mixed genetic background of C57BL/6 and 129/Sv were used in the study. The generation of *Rdh13* KO mice has been described previously (Wang et al., 2012). Mice were allowed free access to food and water, and were housed under specific pathogen-free (SPF) conditions at a constant room temperature of 22–24 °C with a 12 h light-dark cycle during the study. All animal experiments were approved by the Animal Use and Care Committee of Shanghai Jiao Tong University School of Medicine.

2.2. CCl_4-induced liver injury

CCl_4 was used to induce liver injury and liver fibrosis. For acute CCl_4-induced liver injury, a single dose of CCl_4 (1.0 ml/kg body weight of 20% CCl_4 diluted in olive oil) was administered intraperitoneally to WT and *Rdh13* KO mice (n = 4 per group for each time point, 2 male and 2 female mice). Animals were sacrificed 0, 24, 48 and 72 h after CCl_4 injection. In the chronic injury model, WT and *Rdh13* KO mice (n = 10 per group,5 male and 5 female mice) were administered intraperitoneally with the same dose of CCl_4 as acute liver injury twice per week for 4 weeks. Animals were sacrificed three days after the final CCl_4 injection. At the time of sacrifice, mice were anesthetized and liver samples were harvested, rapidly washed with ice-cold phosphate buffered saline (PBS) and dissected into smaller pieces. Liver tissues from different lobes were fixed in 10% neutral formalin for 24 h and then embedded in paraffin for histological examination. Other parts were snap-frozen in liquid nitrogen and stored at −80 °C for RNA and protein analysis.

2.3. Histological analysis

Formalin-fixed liver samples were processed, and paraffin-embedded liver tissue sections (5 μm) were stained with hematoxylin and eosin (H&E) and examined by immunohistochemistry. Liver fibrosis was assessed by means of Masson's trichrome staining for hepatic collagen deposition. Fibrosis deposition was quantified and analyzed by digital imaging with a Zeiss Axioplan 2 imaging analysis system.

2.4. Immunohistochemistry

Formalin-fixed paraffin embedded liver sections (5 μm) were deparaffinized with xylene, hydrated in decreasing concentrations of ethanol. Protein epitopes were unmasked by citrate buffer incubation at 92–98 °C for 15 min. After heat treatment, the sections were allowed to cool at room temperature. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide in PBS for 10 min. Sections were incubated with serum-containing blocking reagents (Vector Laboratories, lnc. Burlingame, CA) for 1 h, then incubated with primary antibody overnight at 4 °C. The staining procedure was performed according to the manufacturer's instructions of VECTASTAINABC system (Vector Labs). Finally, sections were dehydrated and preserved using permount mounting medium and glass cover-slips. Rabbit anti-collagen I (II) (Abcam), rabbit anti-α-SMA (Abcam) and rabbit anti-TIMP-1 (Proteintech Group) antibodies were used.

2.5. RNA isolation and quantitative real-time PCR

Total RNA was isolated from frozen liver tissues using Trizol reagent according to the manufacturer's protocol (Invitrogen). RNA concentration and purity were measured by ultraviolet absorbance. RNA (1 μg) was reverse-transcribed using PrimeScript II 1st Strand cDNA Synthesis Kit (Takara). Quantitative PCR was carried out with SYBR Green real-time PCR Master Mix (Takara). The reactions were performed on an Applied Biosystems 7900HT real-time PCR System, and the cycling parameters were as follows: 95 °C for 5 min and then 42 cycles of 95 °C for 15 s, 58 °C for 15 s and 72 °C for 25 s, followed by a melting curve analysis. Samples were run in triplicate, and relative expression values were normalized to housekeeping gene Gapdh and calculated by the formula: $2^{-\Delta\Delta Ct}$. The primer sequences were shown in Table S1.

2.6. Western blotting

Frozen liver tissues were homogenized in NETN buffer (0.5% Nonidet P-40, 1 mM EDTA, 100 mM NaCl, and 20 mM Tris (pH 8.0)) containing complete Mini EDTA-free Protease Inhibitor Cocktail (Roche) with a homogenizer and sonicator at 4 °C. The samples were centrifuged at 12,000 rpm at 4 °C for 30 min. The supernatants were transferred to new tubes and quantified by Bio-Rad DC Protein Assay. Equal amounts of proteins were denatured, separated on 10% SDS-PAGE and transferred to nitrocellulose transfer membranes (Bio-Rad) which were blocked with 5% non-fat milk (diluted in PBS) for 1 h at room temperature. The membranes were incubated with the primary antibodies overnight at 4 °C under shaking conditions, washed three times with PBST, and then incubated with appropriate fluorescence-conjugated secondary antibodies for 2 h at room temperature. Finally, the