Activated farnesoid X receptor attenuates apoptosis and liver injury in autoimmune hepatitis

FAN LIAN[1], YU WANG[2], YOUJUN XIAO[1], XIWEN WU[3], HANSHI XU[1], LIUQIN LIANG[1] and XIUYAN YANG[1]

Departments of [1]Rheumatology and Clinical Immunology, and [2]Interventional Oncology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong 510080; [3]Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong 510080, P.R. China

Received September 1, 2014; Accepted July 7, 2015

DOI: 10.3892/mmr.2015.4159

Abstract. Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease associated with interface hepatitis, the presence of autoantibodies, regulatory T-cell dysfunction and raised plasma liver enzyme levels. The present study assessed the hepatoprotective and antiapoptotic role of farnesoid X receptor (FXR) in AIH. A mouse model of AIH was induced by treatment with concanavalin A (ConA). The FXR agonist, chenodeoxycholic acid (CDCA), was administered to mice exhibiting ConA-induced liver injury and a normal control. Blood samples were obtained to detect the levels of aminotransferases and inflammatory cytokines. Liver specimens were collected, and hematoxylin-eosin staining was used for histopathological examination and detection. Apoptosis was evaluated using the terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) method. The expression levels of apoptosis-associated genes and proteins were determined by reverse transcription-quantitative polymerase chain reaction and western blotting, respectively. The results demonstrated that FXR was downregulated at the mRNA and protein level in the liver specimens of mice induced with ConA-induced hepatitis. Increased levels of aminotransferases and inflammatory cytokines, including interferon-γ, tumor necrosis factor-α, interleukin (IL)-4 and IL-2, were detected in ConA-treated mice. The mice pretreated with the FXR agonist, CDCA, were more resistant to ConA hepatitis, as indicated by reduced levels of alanine transaminase/aspartate aminotransferase and aminotransferases. The activation of FXR ameliorated hepatocyte apoptosis, as demonstrated by TUNEL analysis and downregulation of the Fas/Fas ligand, tumor necrosis factor-related apoptosis-inducing ligand and caspase-3. Taken together, FXR activation ameliorated liver injury and suppressed inflammatory cytokines in ConA-induced hepatitis. FXR, therefore, exerts a protective role against ConA-induced apoptosis.

Correspondence to: Dr Yu Wang, Department of Interventional Oncology, The First Affiliated Hospital of Sun Yat-sen University, 58 Zhongshan 2nd Road, Guangzhou, Guangdong 510080, P.R. China
E-mail: yuwangguangzhou@163.com

Key words: autoimmune hepatitis, concanavalin A-induced hepatitis, farnesoid X receptor, apoptosis

Introduction

Autoimmune hepatitis (AIH) is a chronic liver disorder characterized by increased transaminase levels, interface hepatitis, hypergammaglobulinemia and the production of autoantibodies (1-3). The immunological mechanism of AIH remains to be elucidated. A lack of response to treatment may lead to end-stage liver disease (4-6).

The farnesoid X receptor (FXR, or nuclear receptor subfamily 1, group H, member 4), a member of the ligand-activated nuclear receptor superfamily, is highly expressed in the liver, bile duct and gastrointestinal tract (7,8). FXR is a bile acid sensor essential in bile acid, cholesterol and triglyceride metabolism (9). Previous studies suggested that FXR is important in the regulation of immune signaling (10,11). FXR-deficient mice tended to lose the maintenance of homeostasis in their innate immunity responses (12). $FXR^{-/-}$ mice exhibited an enhanced susceptibility to T cell-mediated hepatitis and developed immune-mediated liver damage (13). The activation of FXR reduced the expression of inflammatory cytokines and attenuated animal model colitis (13). The expression level of FXR is increased in Barrett's esophagus and an FXR agonist induced the immune response of Barrett's esophagus (14). Concanavalin A (ConA)-induced hepatitis is a well-established T cell-mediated murine model mimicking human AIH. It is characterized by liver injury, granulocyte infiltration and proinflammatory cytokine overproduction (15,16). In the present study, the underlying mechanism by which the expression of FXR affected ConA-induced AIH was assessed.

Materials and methods

Mice and treatments. Sixty female C57BL/6 mice (age, 8-12 weeks; weight, 20-24 g) were obtained from Shanghai Slac Laboratory Animal, Co., Ltd. (Shanghai, China) and were housed in the animal facility under controlled conditions of 22-24˚C and 55% humidity, with a 12 h day/night cycle and free access to food and water. All experiments were approved by the Animal Experiment Committee of

Sun Yat-sen University, following the Guide for the Care and Use of Laboratory Animals (17). To induce hepatitis, ConA (Sigma-Aldrich, St. Louis, MO, USA) was injected through the tail vein [20 mg/kg in 100 μl pyrogen-free phosphate-buffered saline (PBS)]. Negative controls received the identical dose of PBS (18,19). To investigate the effect of FXR on ConA-induced hepatitis, experimental animals were injected with ConA in the presence or absence of the FXR agonist, chenodeoxycholic acid [CDCA; oral administration for 14 days prior to ConA injection and a chow diet with 1% (w/w) CDCA; Chemos GmbH, Regenstauf, Germany]. Blood samples were obtained after 0, 4, 8, 12, 16 and 24 h for the detection of serum markers. All serum markers were assessed in duplicate.

Measurement of alanine transaminase (ALT) and aspartate aminotransferase (AST). Serum ALT and AST levels were analyzed using an ALT/AST R1 and R2 kit (Sekisui Chemical Co., Ltd., Osaka, Japan) with an Automated Chemical Analyzer (7600; Hitachi, Tokyo, Japan), according to the manufacturer's instructions. The concentrations of ALT and AST were expressed as U/liter.

Cytokine detection. The cytokines, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin (IL)-4 and IL-2 in the serum were analyzed by fluorimetry using a Bio-Plex Suspension Array system (cytokine assay), according to the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The total RNA was extracted from mouse liver samples and first-strand DNA was synthesized using the following hexamer primers: Murine FRX forward, 5'-TCCAGGGTTTCAG-ACACTGG-3', and reverse, 5'-GCCGAACGAAGAAACATGG-3' (Guangzhou RiboBio Co., Ltd., Guangzhou, China). and SuperScript II Reverse Transcriptase (Invitrogen Life Technologies, Carlsbad, CA, USA). qPCR was performed using the SYBR® Green PCR Master mix (Applied Biosystems, Applera Deutschland GmbH, Darmstadt, Germany) in a thermocycler ABI PRISM TM7700. The PCR reaction conditions used for all assays were as follows: 95˚C for 30 sec, followed by 40 cycles of amplification at 95˚C for 5 sec, 59˚C for 30 sec and 72˚C for 30 sec. The primer sequence for FXR was designed, as previously described (20,21). RT-qPCR was used to detect the mRNA expression levels of FXR and the apoptotic markers, Fas, Fas ligand (FasL), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), Bcl-2 and caspase-3. β-actin was used as an endogenous control.

Western blot analysis. The total protein was extracted from the mouse liver. An equal quantity of protein from each sample was separated on 10% SDS-PAGE and transferred onto a nitrocellulose membrane (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The membranes were then incubated wtih 5% non-fat milk and 0.1% Tris-buffered saline with Tween 20 blocking solution for 2 h at room temperature. Western blot analysis was performed using rabbit monoclonal anti-mouse FXR (cat. no. NBP2-16550; 1:1,000; Cell Signaling Technology, Frankfurt, Germany) and rabbit monoclonal anti-mouse caspase-3 (cat. no. 9664; 1:1,000; Cell Signaling Technology) antibodies. To control the sample loading, the membranes were stripped with Western Blot stripping buffer (Santa Cruz Biotechnology, Inc., Dallas, TX, USA), and re-probed with mouse monoclonal anti-mouse β-actin antibody (cat. no. A2228; 1:5,000; Sigma-Aldrich). The blots were incubated with enhanced chemiluminescence substrate (Thermo Scientific, Rockford, IL, USA) and the signals were quantified using a Bio-Rad 2000 gel imaging system with Quantity One software (Bio-Rad Laboratories).

Terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) assay. A TUNEL assay was performed using an *in situ* cell death detection kit (Roche, Penzberg, Germany) to observe the apoptotic cells. According to the manufacturer's instructions, paraffin-embedded liver sections were dewaxed by heating the sections to 600˚C prior to washing them with xylene (Shanghai Baoman Biotechnology Co., Ltd., Shanghai, China), and rehydrated through a graded series of ethanol (100, 95, 90, 80 and 70%) and double distilled water. Following permeabilization and PBS washing, the sections were incubated in 50 μl TUNEL reaction mixture for 1 h at 37˚C. The slides were stained with 3,3'-diaminobenzidine following sample quality evaluation. The percentage of TUNEL-positive cells was quantified in randomly selected fields (at least 1,000 liver cells; 5 fields per slide). A total of 1% hematoxylin (Sigma-Aldrich) was used as a counterstain. The cells were visualized using an optical microscope (BX51; Olympus, Tokyo, Japan), and the index was calculated according to a previous report (22): No. apoptotic cells / (no. apoptotic cells + no. negative cells).

Histology and immunohistochemistry. The liver tissues were fixed with 4% paraformaldehyde (Shanghai Standard Co., Ltd., Shanghai, China) for 24 h at 40˚C, and embedded in paraffin. The 5 μm slices were subsequently stained with hematoxylin-eosin and evaluated for pathological changes under a BX51 optical microscope (Olympus). Immunohistochemical staining was performed on the 4 μm paraffin-embedded sections. The slices were incubated with the previously mentioned primary antibody against FXR (1:100 dilution; Cell Signaling Technology). Following incubation with goat anti-rabbit immunoglobulin (Ig) G horseradish peroxidase-conjugated (cat. no. sc-2004; 1:1,000; Santa Cruz Biotechnology, Inc.) and goat anti-mouse IgG horseradish peroxidase-conjugated (cat. no. AP308P; 1:2,000; Sigma-Aldrich) secondary antibodies, the images were visualized using a BX51 optical microscope (Olympus). The positively stained tissue was counted using Image Pro Plus software, version 6.0 (Media Cybernetics, Silver Spring, MD, USA).

Statistical analyses. The data were expressed as the mean ± standard deviation, and all statistical analyses were performed using SPSS 19.0 software (SPSS, Inc., Armonk, NY, USA). The differences in measurements were compared using one-way analysis of variance to compare the mean of the results between groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

ConA induced hepatitis and the expression of FXR. Hepatic histopathology was assessed, as indicated above. Liver injury

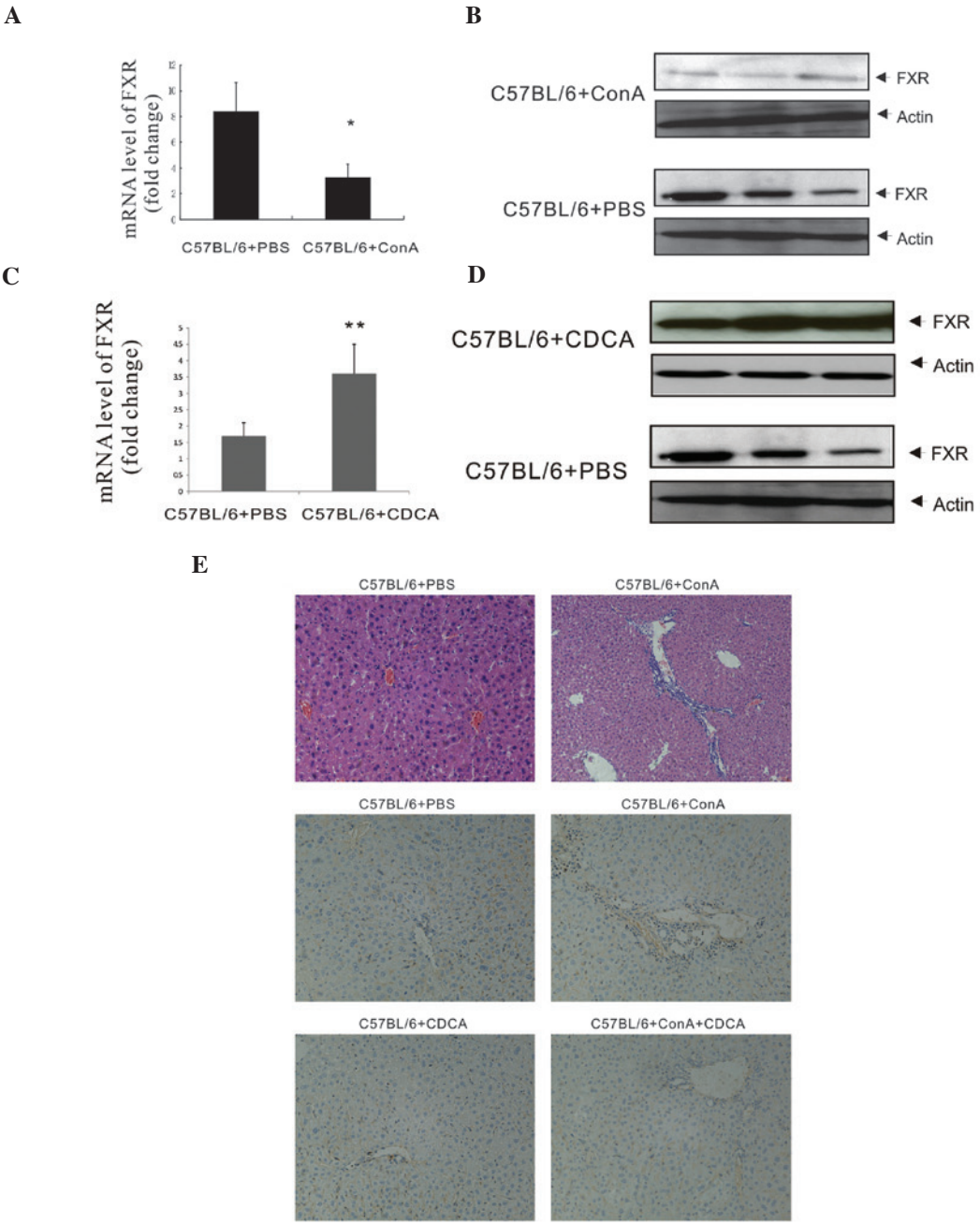

Figure 1. The expression of FXR in ConA-induced hepatitis. (A) The mRNA expression of FXR in ConA-induced hepatitis and normal controls was detected by reverse transcription-quantitative polymerase chain reaction. (B) The protein expression of FXR in ConA-induced hepatitis and normal controls was detected by western blotting. (C) The mRNA expression of FXR in CDCA-treated hepatitis and normal controls was detected by reverse transcription-quantitative polymerase chain reaction. (D) The protein expression of FXR in CDCA-treated hepatitis and normal controls was detected by western blotting. (E) Hematoxylin-eosin staining (original magnification x100) of paraffin-embedded liver sections of ConA-induced hepatitis and normal controls. Immunohistochemical staining (original magnification x200) for the detection of FXR. *P<0.05, ConA-induced hepatitis vs. normal controls, and **P<0.05, C57BL/6+CDCA mice vs. C57BL/6+PBS. CDCA, chenodeoxycholic acid; ConA, concanavalin A; FXR, farnesoid receptor X; PBS, phosphate-buffered saline.

induced by ConA injection was characterized by hepatocellular necrosis, portal inflammation, mononuclear cell infiltration into the parenchyma and sinusoidal hyperemia. The liver structures of the untreated mice were normal. Mice, which were administered CDCA alone, developed no liver injury. As shown in Fig. 1, the expression of FXR was detected in the liver of all mice. CDCA treatment increased the expression of FXR and FXR was demonstrated to be present in smaller quantities in mice inflicted with ConA-induced hepatitis (Fig. 1).

Activation of FXR attenuates ConA-induced liver injury. A total of 15 C57BL/6 mice were fed a chow diet enriched with 1% (w/w) CDCA to stimulate the expression of FXR 1 week prior to ConA injection. Another 15 C57BL/6 mice were fed a normal diet. All the mice were subsequently injected with ConA (20 mg/kg) through their tail veins. The two control groups comprised mice on a CDCA diet without ConA injection, or mice on normal food injected with PBS. Two of the mice fed a normal diet died within 24 h of the ConA injection,

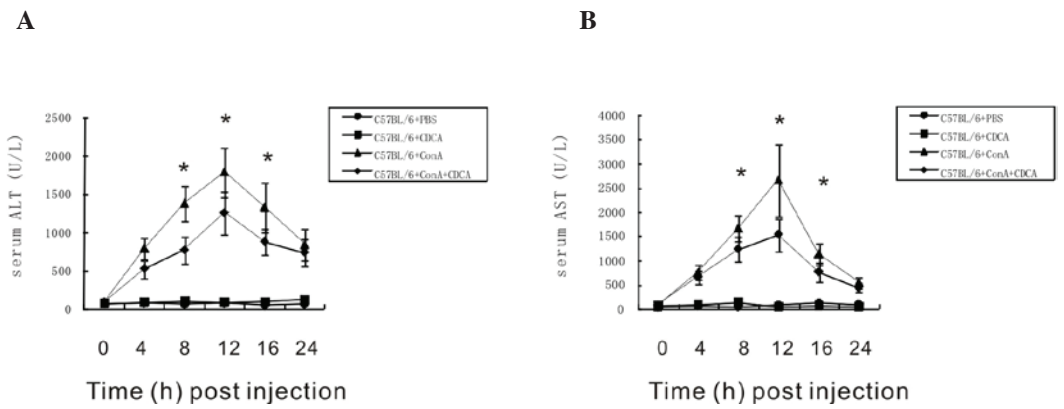

Figure 2. Activation of FXR decreased the serum levels of ALT and AST. (A) The serum levels of ALT and (B) AST were measured in the ConA-treated mice and normal controls in the presence or absence of CDCA at various time points. *P<0.05, ConA-induced hepatitis in the presence of CDCA, vs. ConA-induced hepatitis without CDCA. ALT, alanine transaminase; AST, aspartate aminotransferase; CDCA. CDCA, chenodeoxycholic acid; ConA, concanavalin A; FXR, farnesoid receptor X; PBS, phosphate-buffered saline.

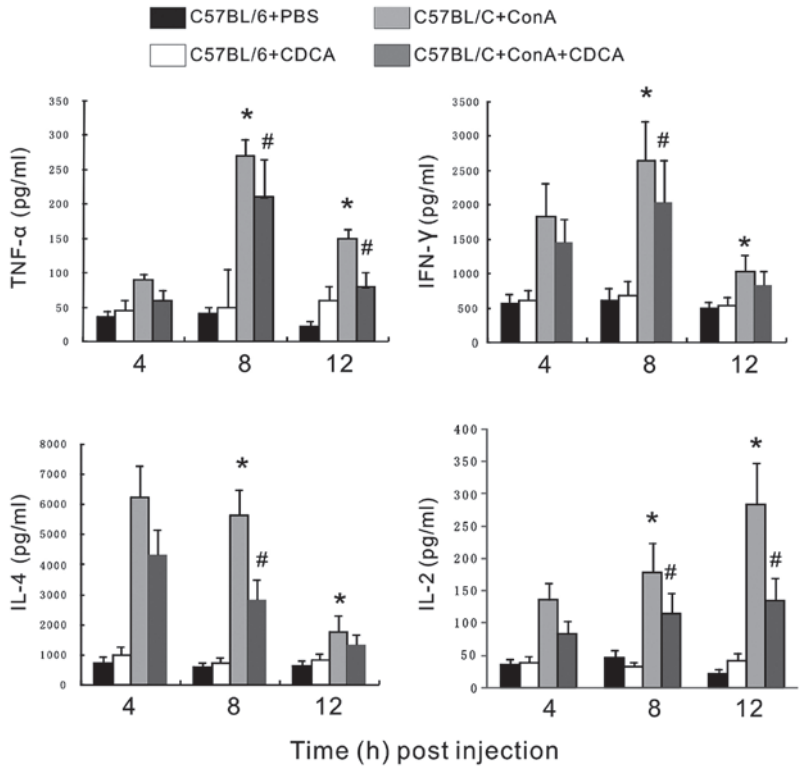

Figure 3. Activation of FXR suppressed inflammatory cytokines in ConA-induced hepatitis. Serum levels of IFN-γ, TNF-α, IL-4 and IL-2 were measured in ConA-treated mice and normal controls in the presence or absence of CDCA at different time points. #P<0.05, ConA-induced hepatitis in the presence of CDCA, vs. ConA-induced hepatitis without CDCA; *P<0.05, ConA induced hepatitis without CDCA, vs. control. CDCA, chenodeoxycholic acid; ConA, conconavalin A; FXR, farnesoid receptor X; IFN-γ, interferon-γ; IL, interleukin, TNF-α, tumor necrosis factor-α; PBS, phosphate-buffered saline.

and all mice on the CDCA diet survived. No controls died within the experimental period.

To assess ConA-induced liver injury between the two groups with or without FXR activation, the level of aminotransferase enzymes was measured. No appreciable level of liver enzyme was identified at the baseline among any of the experimental or control groups; however, increased ALT and AST activities were detected in the ConA-treated mice. The increase in aminotransferase activity peaked at ~12 h following ConA injection, prior to subsiding. The liver enzyme levels were increased in each group treated with ConA, although they were more significantly increased in the group without CDCA treatment (Fig. 2; P<0.05).

Activation of FXR suppresses the inflammatory cytokines induced by ConA. To determine the effect of FXR on inflammatory responses, the serum cytokine levels of IFN-γ, TNF-α, IL-4 and IL-2 were measured. A significant increase (P<0.05) in the level of the inflammatory cytokines was identified following ConA injection, particularly in the mice fed a normal diet. The levels of these cytokines were reduced by CDCA treatment (Fig. 3).

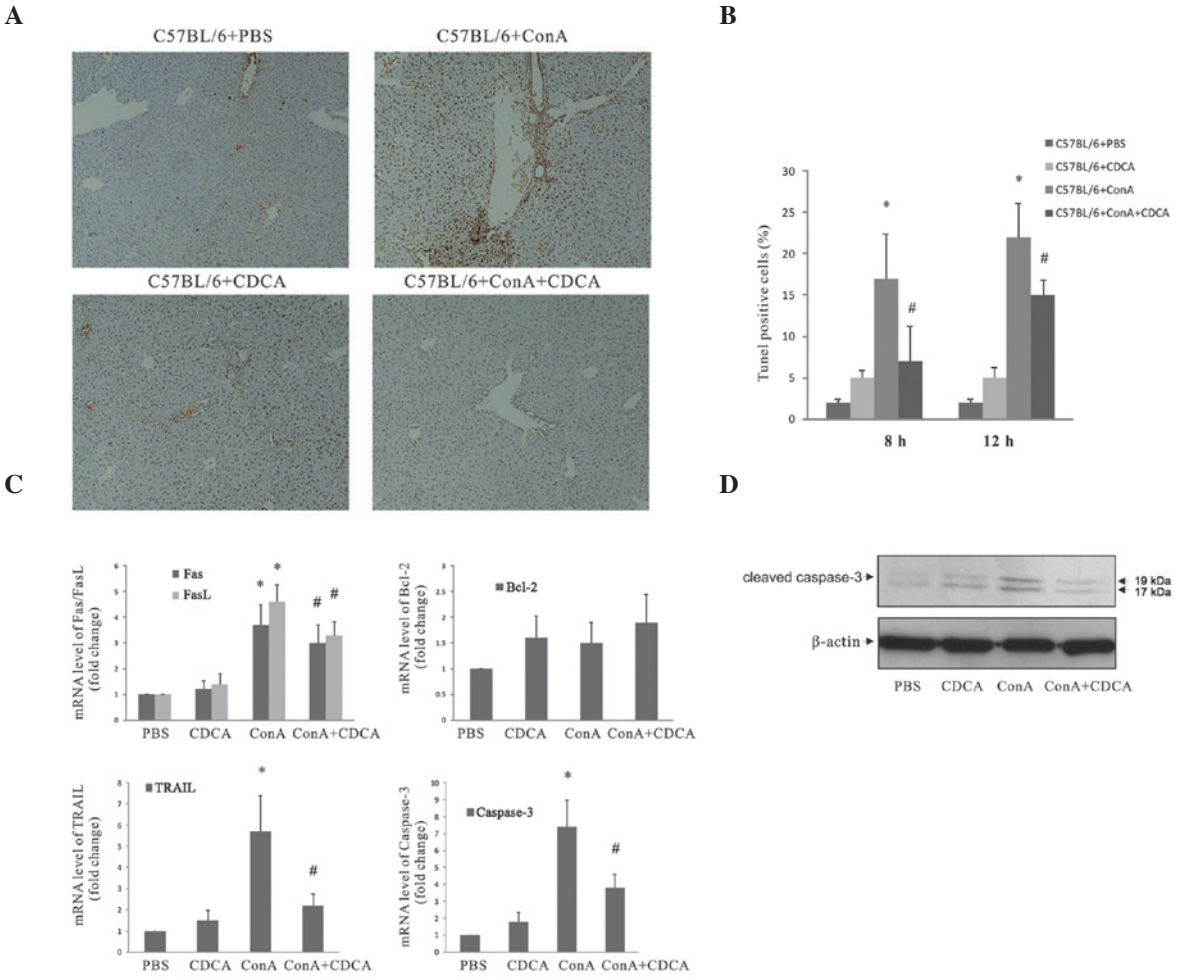

Figure 4. Activation of FXR reduced hepatic apoptosis. (A) Representative images of the TUNEL assay (original magnification x100). The cells with dark brown nuclei were considered TUNEL-positive. (B) The ratio of TUNEL-positive cells was calculated and the percentage of TUNEL-positive cells significantly decreased when FXR was activated upon treatment with CDCA. (C) The mRNA expression level of the apoptosis markers, Fas/FasL, Bcl-2, TRAIL and caspase-3 were determined by RT-qPCR. The data are expressed as the mean ± standard deviation. (D) The protein expression of caspase-3 in ConA-induced hepatitis and normal controls was detected by western blotting. $^{\#}$P<0.05, ConA-induced hepatitis in the presence of CDCA, vs. ConA-induced hepatitis without CDCA; *P<0.05, ConA-induced hepatitis without CDCA, vs. control. CDCA, chenodeoxycholic acid; ConA, conconavalin A; FasL, Fas ligand; FXR, farnesoid receptor X; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TUNEL, terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling; PBS, phosphate-buffered saline.

Activation of FXR suppresses ConA-induced apoptosis in the liver. Following ConA injection, hepatic apoptosis was less evident in the C57BL/6 mice pretreated with CDCA compared with the mice without CDCA. TUNEL assays were used to analyze liver sections (Fig. 4A). As shown in Fig. 4B, the TUNEL index was decreased in the livers pretreated with CDCA.

The mRNA expression levels of Fas/FasL, Bcl-2, TRAIL, Bcl-2 and caspase-3 were detected by RT-qPCR. The mRNA expression levels of Fas/FasL, TRAIL and caspase-3 were low in the control groups, although their expression levels were significantly increased following ConA injection (P<0.05). Treatment with CDCA significantly downregulated the mRNA expression levels of Fas/FasL, TRAIL and caspase-3. No significant differences in the level of Bcl-2 protein were identified among the four groups (Fig. 4C). The level of caspase-3 protein detected by western blotting exhibited a similar trend, suggesting that FXR exerts an antiapoptotic role in ConA-induced hepatitis.

Discussion

AIH is a chronic inflammatory liver disease, the cause of which remains to be elucidated. AIH is characterized by increased levels of autoantibodies and immunoglobulins, hepatic inflammation and liver dysfunction (6,23,24). A subset of patients with AIH may gradually progress to liver failure.

FXR is a member of the ligand-activated nuclear receptor superfamily and bile acid sensor, highly expressed in the liver. Previous studies have associated FXR with inflammatory and immune-mediated diseases (25,26). In the present study, to detect any putative protective role of FXR *in vivo*, an experimental mouse model of AIH was established by treatment with ConA. The ConA-induced hepatitis, T cell-activated murine model system, which models several aspects of pathological lesions and mechanisms of pathogenesis in human AIH, has been used as an experimental model for AIH research (16,27). ConA-induced hepatitis typically demonstrates T-cell infiltration and portal inflammation, which results in cytokine

overproduction, hepatocyte apoptosis and necrosis (28-31). On establishing this model, it was identified that the mRNA and protein expression level of FXR was downregulated in ConA-induced hepatitis.

To gain an insight into the biological roles of FXR in the development of AIH, the CDCA FXR agonist, which increases the expression of FXR was used. FXR activation alleviated symptoms of portal inflammation, lipid deposition and focal necrosis. The activity of ALT and AST is widely used for the assessment of liver injury (32). In the present study, the levels of aminotransferases were subsequently measured, and it was demonstrated that FXR protected mice against the development of ConA-induced liver injury by significantly lowering the levels of ALT and AST, suggesting that administration of the FXR agonist may attenuate AIH.

ConA-induced hepatitis is, to a certain extent, regulated by the balance of protective and stimulatory cytokines (33). It is possible that the effects of FXR in counteracting ConA-induced hepatitis are mediated by modulating inflammatory cytokines and it is considered that proinflammatory cytokines are involved in ConA-induced liver damage. Inflammatory mediators, including TNF-α, IFN-γ, IL-4 and IL-2, are essential for the progression of hepatitis, and liver injury was attenuated by IL-6, IL-10 and IL-22. The present data suggested that FXR inhibited the production of inflammatory cytokines and was involved in the inflammatory responses as a negative regulator. TNF-α and IFN-γ are key to the inflammatory hepatocellular apoptosis induced by ConA (34). Mice deficient in tumor necrosis factor receptor 1 (TNFR) 1 and TNFR2 or TNF-α inhibitor are less likely to develop ConA-induced hepatitis (35). The present study demonstrated that the upregulation of TNF-α and IFN-γ occurred following ConA injection, which is compatible with previous reports (36). These changes were reversed in the experimental models by pretreatment with an FXR agonist. The present study suggested that FXR exerts a protective role at the early stage and continues to subsequently have an effect, and therefore may partly prevent hepatocyte necrosis at a late stage. IL-2 and IL-4 are also major cytokines involved in ConA-induced hepatitis (37,38). Similar to previous reports, the serum levels of IL-2 and IL-4 were markedly increased following ConA injection in the present study. These changes were less pronounced in the mice pretreated with CDCA and the results suggested a protective role of FXR against inflammatory liver injury.

Hepatocyte apoptosis is a critical event in the pathogenesis of ConA-induced liver damage. Several apoptosis-associated genes and enzymes are considered to exert important roles in the development of hepatitis (39). Previous reports have linked FXR to the regulation of ConA-induced hepatitis by postulating a mechanism, which focuses on the inhibition of natural killer T (NKT) cell activation (13). ConA-induced activation of NKT cells triggers the upregulation of FasL, which interacts with its death receptor, Fas, on the surface of hepatocytes, leading to apoptosis. It was observed that mice with mutations in the Fas-FasL apoptotic pathway were resistant to hepatitis (40-42). In addition, activated NKT cells are responsible for the increased level of IFN-γ, which is required by NKT cells for the expression of TRAIL, another effector for hepatocyte apoptosis (43). TRAIL is associated with the inflammatory status of the liver (44). Similarly, caspase activation is also involved. Caspase-3, a member of the IL-1β-converting enzyme family and downstream effector, is the key element promoting apoptosis (45). Based on the results of the present study, mice pretreated with the FXR agonist were less sensitive to ConA-induced apoptosis. The TUNEL assay confirmed the protective effect of FXR on apoptosis. FXR activation markedly downregulated Fas/FasL, TRAIL and caspase-3; however, no significant difference in the level of Bcl-2 was observed. These data suggested that the underlying antiapoptotic mechanisms of FXR may be associated with several signaling pathways, including Fas/FasL, TRAIL and caspase-3, and that Bcl-2 was not involved.

Taken together, the present study suggested that FXR was downregulated in ConA-induced hepatitis. FXR activation dramatically attenuated liver injury and suppressed inflammatory cytokines. The protective effect of FXR may depend on suppressing apoptosis through several signaling pathways, including Fas/FasL, TRAIL and caspase-3. The present data corroborate the essential role of FXR in the ConA model of liver injury, and will pave the way for further studies in AIH to target FXR for potential therapeutic alternatives.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (nos. 81102270 and 81101135), the Guangdong Natural Science Foundation (no. S2012010009075) and the Fundamental Research Funds for the Central Universities of China (no. 11ykpy15).

References

1. Vergani D and Mieli-Vergani G: Aetiopathogenesis of autoimmune hepatitis. World J Gastroenterol 14: 3306-3312, 2008.
2. Lapierre P, Béland K, Yang R and Alvarez F: Adoptive transfer of *ex vivo* expanded regulatory T cells in an autoimmune hepatitis murine model restores peripheral tolerance. Hepatology 57: 217-227, 2013.
3. Strassburg CP: Autoimmune hepatitis. Dig Dis 31: 155-163, 2013.
4. Sebode M and Lohse AW: Future perspective: Immunomodulatory therapy for autoimmune hepatitis. Dig Dis 32: 502-506, 2014.
5. Trivedi PJ and Hirschfield GM: Treatment of autoimmune liver disease: Current and future therapeutic options. Ther Adv Chronic Dis 4: 119-141, 2013.
6. Manns MP, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D and Vierling JM; American Association for the Study of Liver Diseases: Diagnosis and management of autoimmune hepatitis. Hepatology 51: 2193-2213, 2010.
7. Wang YD, Chen WD, Moore DD and Huang W: FXR: A metabolic regulator and cell protector. Cell Res 18: 1087-1095, 2008.
8. Lee FY, Lee H, Hubbert ML, Edwards PA and Zhang Y: FXR, a multipurpose nuclear receptor. Trends Biochem Sci 31: 572-580, 2006.
9. Fiorucci S, Rizzo G, Donini A, Distrutti E and Santucci L: Targeting farnesoid X receptor for liver and metabolic disorders. Trends Mol Med 13: 298-309, 2007.
10. Renga B, Mencarelli A, Cipriani S, D'Amore C, Carino A, Bruno A, Francisci D, Zampella A, Distrutti E and Fiorucci S: The bile acid sensor FXR is required for immune-regulatory activities of TLR-9 in intestinal inflammation. PloS One 8: e54472, 2013.
11. McMahan RH, Wang XX, Cheng LL, Krisko T, Smith M, El Kasmi K, Pruzanski M, Adorini L, Golden-Mason L, Levi M, *et al*: Bile acid receptor activation modulates hepatic monocyte activity and improves nonalcoholic fatty liver disease. J Biol Chem 288: 11761-11770, 2013.

12. Fiorucci S, Cipriani S, Mencarelli A, Renga B, Distrutti E and Baldelli F: Counter-regulatory role of bile acid activated receptors in immunity and inflammation. Curr Mol Med 10: 579-595, 2010.
13. Vavassori P, Mencarelli A, Renga B, Distrutti E and Fiorucci S: The bile acid receptor FXR is a modulator of intestinal innate immunity. Journal of immunology (Baltimore, Md. : 1950) 183: 6251-6261, 2009.
14. Capello A, Moons LM, Van de Winkel A, Siersema PD, van Dekken H, Kuipers EJ and Kusters JG: Bile acid-stimulated expression of the farnesoid X receptor enhances the immune response in Barrett esophagus. Am J Gastroenterol 103: 1510-1516, 2008.
15. Tiegs G, Hentschel J and Wendel A: A T cell-dependent experimental liver injury in mice inducible by concanavalin A. J Clin Invest 90: 196-203, 1992.
16. Shao X, Qian Y, Xu C, Hong B, Xu W, Shen L, Jin C, Wu Z, Tong X and Yao H: The protective effect of intrasplenic transplantation of Ad-IL-18BP/IL-4 gene-modified fetal hepatocytes on ConA-induced hepatitis in mice. PloS One 8: e58836, 2013.
17. Institute for Laboratory Animal Research 1996: Guide for the care and use of laboratory animals. Washington (DC). National Academies Press, USA, pp1-141, 1996.
18. Wang HX, Liu M, Weng SY, Li JJ, Xie C, He HL, Guan W, Yuan YS and Gao J: Immune mechanisms of Concanavalin A model of autoimmune hepatitis. World J Gastroenterol 18: 119-125, 2012.
19. Christen U, Holdener M and Hintermann E: Animal models for autoimmune hepatitis. Autoimmun Rev 6: 306-311, 2007.
20. Li J, Pircher PC, Schulman IG and Westin SK: Regulation of complement C3 expression by the bile acid receptor FXR. J Biol Chem 280: 7427-7434, 2005.
21. Xing X, Burgermeister E, Geisler F, Einwächter H, Fan L, Hiber M, Rauser S, Walch A, Röcken C, Ebeling M, *et al*: Hematopoietically expressed homeobox is a target gene of farnesoid X receptor in chenodeoxycholic acid-induced liver hypertrophy. Hepatology 49: 979-988, 2009.
22. Garrity MM, Burgart LJ, Riehle DL, Hill EM, Sebo TJ and Witzig T: Identifying and quantifying apoptosis: Navigating technical pitfalls. Mod Pathol 16: 389-394, 2003.
23. Verma S, Torbenson M and Thuluvath PJ: The impact of ethnicity on the natural history of autoimmune hepatitis. Hepatology 46: 1828-1835, 2007.
24. Verma S, Maheshwari A and Thuluvath P: Liver failure as initial presentation of autoimmune hepatitis: Clinical characteristics, predictors of response to steroid therapy, and outcomes. Hepatology 49: 1396-1397, 2009.
25. Gadaleta RM, van Erpecum KJ, Oldenburg B, Willemsen EC, Renooij W, Murzilli S, Klomp LW, Siersema PD, Schipper ME, Danese S, *et al*.: Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. Gut 60: 463-472, 2011.
26. Wildenberg ME and van den Brink GR: FXR activation inhibits inflammation and preserves the intestinal barrier in IBD. Gut 60: 432-433, 2011.
27. Chen F, Zhu HH, Zhou LF, Li J, Zhao LY, Wu SS, Wang J, Liu W and Chen Z: Genes related to the very early stage of ConA-induced fulminant hepatitis: A gene-chip-based study in a mouse model. BMC Genomics 11: 240, 2010.
28. Zhou Y, Dai W, Lin C, Wang F, He L, Shen M, Chen P, Wang C, Lu J, Xu L, *et al*: Protective effects of necrostatin-1 against concanavalin A-induced acute hepatic injury in mice. Mediators Inflamm 2013: 706156, 2013.
29. Higashimoto M, Sakai Y, Takamura M, Usui S, Nasti A, Yoshida K, Seki A, Komura T, Honda M, Wada T, *et al*: Adipose tissue derived stromal stem cell therapy in murine ConA-derived hepatitis is dependent on myeloid-lineage and CD4+ T-cell suppression. Eur J Immunol 43: 2956-2968, 2013.
30. Xu X, Hu Y, Zhai X, Lin M, Chen Z, Tian X, Zhang F, Gao D, Ma X, Lv L, *et al*: Salvianolic acid A preconditioning confers protection against concanavalin A-induced liver injury through SIRT1-mediated repression of p66shc in mice. Toxicol Appl Pharmacol 273: 68-76, 2013.
31. Arshad MI, Piquet-Pellorce C, L'Helgoualc'h A, Rauch M, Patrat-Delon S, Ezan F, Lucas-Clerc C, Nabti S, Lehuen A, Cubero FJ, *et al*: TRAIL but not FasL and TNFα, regulates IL-33 expression in murine hepatocytes during acute hepatitis. Hepatology 56: 2353-2362, 2012.
32. Zhou YC, Chen S, Cao JJ, Chen SY, Xie YF and Niu QX: Adenovirus-mediated viral interleukin-10 gene transfer prevents concanavalin A-induced liver injury. Dig Liver Dis 44: 398-405, 2012.
33. Godfrey DI, Hammond KJ, Poulton LD, Smyth MJ and Baxter AG: NKT cells: Facts, functions and fallacies. Immunol Today 21: 573-583, 2000.
34. Volarevic V, Mitrovic M, Milovanovic M, Zelen I, Nikolic I, Mitrovic S, Pejnovic N, Arsenijevic N and Lukic ML: Protective role of IL-33/ST2 axis in Con A-induced hepatitis. J Hepatol 56: 26-33, 2012.
35. Küsters S, Tiegs G, Alexopoulou L, *et al*: *In vivo* evidence for a functional role of both tumor necrosis factor (TNF) receptors and transmembrane TNF in experimental hepatitis. Eur J Immunol 27: 2870-2875, 1997.
36. Sass G, Heinlein S, Agli A, Bang R, Schümann J and Tiegs G: Cytokine expression in three mouse models of experimental hepatitis. Cytokine 19: 115-120, 2002.
37. Takahashi K, Murakami M, Kikuchi H, Oshima Y and Kubohara Y: Derivatives of Dictyostelium differentiation-inducing factors promote mitogen-activated IL-2 production via AP-1 in Jurkat cells. Life Sci 88: 480-485, 2011.
38. Miller ML, Sun Y and Fu YX: Cutting edge: B and T lymphocyte attenuator signaling on NKT cells inhibits cytokine release and tissue injury in early immune responses. Journal of immunology (Baltimore, Md. : 1950) 183: 32-36, 2009.
39. Kahraman A, Gerken G and Canbay A: Apoptosis in immune-mediated liver diseases. Dig Dis 28: 144-149, 2010.
40. Zhang Y, Shan L, Hua Y, Wang D, Zeng H, Liu R, Zhang W and Hu Z: Baicalein selectively induces apoptosis in activated lymphocytes and ameliorates concanavalin a-induced hepatitis in mice. PloS One 8: e69592, 2013.
41. Leist M, Gantner F, Bohlinger I, Tiegs G, Germann PG and Wendel A: Tumor necrosis factor-induced hepatocyte apoptosis precedes liver failure in experimental murine shock models. Am J Pathol 146: 1220-1234, 1995.
42. Stassi G, Di Felice V, Todaro M, Cappello F, Zummo G, Farina F, Trucco M and De Maria R: Involvement of Fas/FasL system in the pathogenesis of autoimmune diseases and Wilson's disease. Arch Immunol Ther Exp (Warsz) 47: 129-133, 1999.
43. Peppa D, Micco L, Javaid A, Kennedy PT, Schurich A, Dunn C, Pallant C, Ellis G, Khanna P, Dusheiko G, *et al*: Blockade of immunosuppressive cytokines restores NK cell antiviral function in chronic hepatitis B virus infection. PLoS Pathog 6: e1001227, 2010.
44. Beraza N, Malato Y, Sander LE, Al-Masaoudi M, Freimuth J, Riethmacher D, Gores GJ, Roskams T, Liedtke C and Trautwein C: Hepatocyte-specific NEMO deletion promotes NK/NKT cell- and TRAIL-dependent liver damage. J Exp Med 206: 1727-1737, 2009.
45. Gao D, Xu Z, Qiao P, Liu S, Zhang L, He P, Zhang X, Wang and Min W: Cadmium induces liver cell apoptosis through caspase-3A activation in purse red common carp (*Cyprinus carpio*). PloS One 8: e83423, 2013.