Acanthoic acid, a diterpene in *Acanthopanax koreanum*, ameliorates the development of liver fibrosis via LXR signals
Ting Bai 1, You-li Yao 1, Xue-Jun Jin, Li-hua Lian, Qian Li, Ning Yang, Quan Jin, Yan-ling Wu *, Ji-xing Nan *

Key Laboratory for Natural Resource of Changbai Mountain & Functional Molecules, Ministry of Education, College of Pharmacy, Yanbian University, Yanji 133002, Jilin Province, China

A R T I C L E   I N F O

Article history:
Received 16 October 2013
Received in revised form 21 March 2014
Accepted 24 April 2014
Available online 5 May 2014

Keywords:
*Acanthopanax koreanum* Nakai (Araliaceae)
Acanthoic acid (AA)
Carbon tetrachloride (CCl4)
Liver fibrosis
Liver X receptors (LXRs)

A B S T R A C T

Liver X receptors (LXRs)-mediated signals in acanthoic acid (AA) ameliorating liver fibrosis were examined in carbon tetrachloride (CCl4)-induced mice and TGF-β stimulated hepatic stellate cells (HSCs). AA was isolated from the root of *Acanthopanax koreanum* Nakai (Araliaceae). CCL4-treated mice were intraperitoneally injected with 10% CCL4 in olive oil (2 mL/kg for 8 weeks). In AA treated groups, mice were stimulated hepatic stellate cells (HSCs). AA could inhibit the expression of LXR ligands in immortalized and primary stellate cells from mice, also in carbon tetrachloride-treated and LXRβ-/- mice. They found that LXR signaling was a determinant of stellate cell activation and susceptible to fibrotic liver disease. And

1. Introduction

Various factors could induce chronic liver injury, including alcohol abuse, viral infection, cholestasis, fat accumulation and autoimmune diseases [1]. The common result of chronic liver injury might be liver fibrosis, a wound-healing response to these stimuli. Liver fibrosis is the key joint during chronic liver injury to cirrhosis and even hepatoma, which could be regarded as herald stage of cirrhosis. Once cirrhosis develops, it is difficult to recover ordinary state, and carries a high risk of morbidity and mortality. Liver fibrosis results in the deposition of scar tissue and excessive production of the extracellular matrix (ECM) [2]. In general, there is a fine balance between synthesis and degradation of ECM. Matrix metalloproteinase (MMPs) would promote the degradation of ECM, while tissue inhibitor of metalloproteinase 1 (TIMP-1) inhibits the activities of MMPs to prevent the degradation of ECM [3]. Therefore the TIMP/MMP balance is critical for ECM metabolism. Quiescent hepatic stellate cells (HSCs) express low levels of ECM. Upon activations, these cells present a myofibroblastic phenotype and contribute excessive deposition of ECM, such as collagens, hyaluronic acid, fibronectins [4]. Activated HSCs express excessive α-smooth muscle actin (α-SMA) and collagen I with high proliferative activity, and secrete pro-inflammatory cytokines and chemotactic cytokines [5]. Therefore, it is essential to develop therapeutic strategies to inhibit liver fibrosis and inflammation at the same time.

Liver X receptors (LXRs) are nuclear receptors of transcription factors and play important roles in lipid metabolism and anti-inflammatory signaling [6]. These receptors occur in two isoforms, LXRα and LXRβ, and functions as ligand-activated transcription factors [7]. Current researches indicate that elevated intracellular cholesterol activates LXRs in multiple cells. Activated LXRs would be involved in cholesterol absorption, efflux, transport, excretion, and modulate immune and inflammatory response [8]. Synthetic LXR agonists promote cholesterol efflux and inhibit inflammation in vivo and inhibit the development of atherosclerosis in animal models [9]. Thus, LXRs are considered as attractive targets in human metabolic disease aiming for integration of lipid metabolic and inflammatory signaling intervention. Beaven et al. detected the expression of LXR ligands in immortalized and primary stellate cells from mice, also in carbon tetrachloride-treated and LXRβ-/- mice [10]. They found that LXR signaling was a determinant of stellate cell activation and susceptible to fibrotic liver disease. And
they convincingly demonstrated that LXR displayed antifibrogenic properties via a direct LXRα–dependent effect on activated hepatic stellate cells. These results reveal an unexpected role for LXRs signaling in the modulation of hepatic stellate cell function [11]. Therefore, it is interesting to reveal the exact effect of LXRs in the development of liver fibrosis.

Acanthoic acid (AA), (−)-primara-9(11), 15-dien-19-oic acid, is a pimariendane diterpene, isolated from the root bark of Acanthopanax koreanum Nakai (Araliaceae) that is widely used as food or medicine in Asia. It was found that AA suppressed the production of TNF-α, and interleukin-1 (IL-1), and interleukin-8 (IL-8) [12,13]. And AA could induce cell apoptosis through activation of the p38 MAPK pathway in HL-60 cells [14]. In our previous research, we detected the protective effect of AA in D-GalN/LPS or acetaminophen-induced fulminant liver failure in mice [15–17]. And AA showed a protective effect by down-regulation of TNF-α secretion. AA decreased serum aminotransferase levels and ameliorated oxidant induced hepatotoxicity caused by APAP. We also found that AA could regulate the expression of hypoxia inducible factor-1α (HIF-1α) expression to prevent APAP-induced hepatotoxicity. Therefore, we currently evaluated the ability of AA to prevent the development and progression of carbon tetrachloride (CCL4)-induced liver fibrosis in mice, which is more similar with human liver fibrosis [18,19]. This study focused on the molecular mechanisms underlying the effects of AA in this model via LXRs signaling pathway.

2. Materials and methods

2.1. Plant material

A. koreanum Nakai (Araliaceae) was afforded by Yanbian University, purchased from Changbai country Jilin province and was authenticated by Prof. Hui-Zi Lv of the College of Pharmacy, Yanbian University. A voucher specimen (YBUCP201001) was deposited in the Herbarium of College of Pharmacy, Yanbian University, China.

2.2. Extraction of acanthoic acid

Acanthoic Acid (AA) was isolated from the roots of A. koreanum Nakai (Araliaceae) as described previously [20]. In brief, air dried powder from the roots of A. koreanum Nakai (Araliaceae) (1000 g) was extracted with methanol 80% under reflux for 15 h at room temperature several times until exhaustion by maceration method. After evaporating solvent, the residue suspended in distilled water was extracted with methylene chloride. The extract was concentrated under reduced pressure of crude extract. The extract 50 g was subjected to silica gel column chromatography eluting with hexane–ethylacetate gradient system (20:1–1:1) gradually. The fractions that showed similar thin layer chromatography (TLC) were collected. Finally, acanthoic acid (AA) (0.82% yield; >95% purity) was obtained by a paraparticulate HPLC using a J'sphere ODS-H80 column (YMC Co., Ltd., Kyoto, Japan) eluted with methanol, and then purified on Sephadex LH-20 column using methanol and water as eluents.

2.3. Animals and treatment

Male C57BL/6 mice were obtained from the Animal Division of Jilin University (Jilin, China). The mice were housed in the animal quarters at the College of Pharmacy of Yanbian University. They were maintained at 22 °C on a 12:12 h light–dark cycle, and they had free access to rodent chow and tap water. The experimental procedures were approved by the Institutional Animal Care and Use Committee of Yanbian University. Mice were randomly divided into five groups randomly, including normal group, AA (50 mg/kg) single group, CCL4-treated group, AA (20 mg/kg) plus CCL4-treated group, and AA (50 mg/kg) plus CCL4-treated group. AA was suspended initially in 2% (v/v) Tween 80 and then further in saline. CCL4-treated mice were intraperitoneally injected with 10% CCl4 in olive oil (2 mL/kg body weight, twice a week for 4 weeks and then once a week for the next 4 weeks). In AA (20 mg/kg) plus CCL4-treated group and AA (50 mg/kg) plus CCL4-treated group, mice were intragastrically administrated with AA (20 or 50 mg/kg) 3 times per week for 8 weeks simultaneously with CCl4. And the 3 times per week for AA were on Monday, Wednesday, and Friday morning. Dosages of AA were selected according to previous study, and LD50 of AA (mouse) was 300 mg/kg provided by Clear-synth Labs Pvt. Ltd. (India). Mice were sacrificed at 8 weeks and then blood samples were taken for serum biochemistry. The liver was dissected, weighed, and snap frozen in liquid nitrogen, and then stored at −80 °C until analyzed.

2.4. Determination of serum transaminases levels

Blood was collected at 4 h after the last CCl4 administration. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and serum TNF-α levels were detected by using an Autodry Chemistry Analyzer (SPOTCHEM SP4410, Arkray, Japan).

2.5. Histological examination

Liver tissues were fixed in 10% buffered formalin, processed with graded volumes of alcohol, embedded in paraffin, sectioned to a thickness of 4 μm and stained with hematoxylin and eosin for histological studies according to the instruction. Slides were stained with hematoxylin, mounted and observed by light microscopy and examined in a blind fashion. Slides were stained with Masson’s trichome method to determine collagen deposition in liver tissue. α-Smooth muscle actin (α-SMA) was assessed immuno-histochemically by the strepavidin–biotin–peroxidase complex method, using the LSAB®2 Kit (DAKO Co., Carpinteria, USA) and anti-α-smooth muscle actin monoclonal antibody (Boehringer Mannheim, Germany).

2.6. Cell cultures

HSC-T6, an immortalized rat hepatic stellate cell line, was kindly provided by Dr. Jung-Joon Lee (Center for Molecular Cancer Research, Korea Research Institute of Bioscience and Biotechnology, Ochang, Chungbuk, Republic of Korea). HSC-T6 were incubated in DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C under 5% CO2. The cultures were passaged by trypsinization every 3 day and cells were plated in 100 mm culture dishes at a density of 1 × 106 cells per dish in DMEM. For the experiment, cells were exposed to TGF-β with 0.1% fetal bovine serum in the absence or presence of AA at the indicated concentrations.

2.7. The effects of acanthoic acid on HSC-T6 cells

Firstly, in order to detect cytotoxicity of AA on HSC-T6 cells, we selected different concentrations (0–200 μmol/l) of AA to stimulate HSC-T6 cells. Cells were incubated in 96 well plates (1 × 104 cells/well). After 24 h of incubation, new medium containing MTT (5 μg/l) was replaced. The plates were incubated for 3 h and then dissolved in DMSO. The absorbance at 570 nm was recorded using a microplate reader.

Secondly, cells were cultured in six-well plates (5 × 105 cells/ml, 3 ml/well) under a humidified 5% CO2 atmosphere at 37 °C to
over 80% confluence. TGF-β (5 ng/ml) was added to activate HSC-T6 cells for 2 h, and then treated with AA (1, 3, or 10 µmol/l) for 24 h before analysis. After the culture medium was removed, the cells were washed in phosphate-buffered saline (PBS) and lysed in 50 µmol/l Tris–HCl, pH 7.5, 150 mmol/l NaCl, 1% Triton X-100, 0.1 mmol/l Na3VO4, 1 mmol/l PMSF and 0.1 mmol/l aprotinin. Cell debris was removed by centrifugation at 15,000 g for 30 min at 4 °C. Protein concentrations were determined by the BioRad protein assay.

2.8. Western blot analysis

After treatments, the liver tissue or cells were collected and lysed with lysis buffer. 20–100 µg of protein from freshly prepared liver homogenate fractions or cell lysate was performed Western blot analysis to detect α-SMA, Collagen-I, MMP-13, TIMP-1, LXRα, LXRβ, NF-κB and IκB-α expression. Blots were scanned and bands were quantified with Quantity One software (Bio-Rad, USA).

2.9. Statistical analysis

The results are expressed as the mean ± SD statistical differences were determined by one-way analysis of variance (ANOVA) and Tukey’s multiple comparison tests. Calculations were performed using the GraphPad Prism program (GraphPad Software, Inc., San Diego, CA, USA).

3. Results

3.1. Acanthoic acid ameliorated the biochemical levels in CCl4-treated mice

The chemical structure of AA is shown in Fig. 1A. Biochemical analyses of serum enzymes were performed to verify the role of AA in the protection of liver treated with CCl4. As shown in Fig. 1B, administration of CCl4 for 8 weeks induced serum ALT and AST elevation (465 ± 43 and 891 ± 76 IU/L, respectively), which significantly higher than that in normal group (32 ± 8 pg/mL) (p < 0.01). Single AA administration significantly attenuated the effects on ALT and AST levels compared with normal group (41 ± 9 and 45 ± 6 IU/L, respectively), which indicated that AA (50 mg/kg) was safe for mice, at least without hepatotoxicity under this dosage. However, ALT and AST levels in CCl4-treated mice were significantly decreased by both low- and high-dose AA in a dose-dependent manner (237 ± 21 and 426 ± 49 IU/L in CCl4 + AA-20 group, and 113 ± 16 and 239 ± 34 IU/L in CCl4 + AA-50 group, respectively) (p < 0.01).

To investigate whether AA represses the production of proinflammatory cytokine, which play central role in inflammatory disease, TNF-α level was detected in mice stimulated with CCl4 in the presence or absence of AA. After treatment with CCl4, serum TNF-α level (152 ± 44 pg/mL) significantly increased compared with that in normal group (32 ± 8 pg/mL) (p < 0.01). Pre-treatment with AA resulted in a significant decrease in cytokine production (Fig. 1C) (116 ± 36 pg/mL in CCl4 + AA-20 group, and 81 ± 20 pg/mL in CCl4 + AA-50 group, respectively) (p < 0.05). Single AA administration also did not affect TNF-α levels compared with normal group (43 ± 7 pg/mL).

3.2. Effects of acanthoic acid on histopathological analysis and immune staining in CCl4-treated mice

Histopathological analysis revealed that CCl4 induced lipid accumulation, degeneration, fibrosis, and necrosis in CCl4-treated livers (Fig. 2B) when compared to normal livers (Fig. 2A). All mice except normal group exhibited different extent of the ballooning degeneration in the centrolobular zone and the necrosis of hepatocytes. The CCl4-induced damage developed more severely than other groups treated with AA plus CCl4. Both low and high doses of AA attenuated the morphologic changes (Fig. 2C, D), significantly decreased CCl4-induced immune cell infiltration and fibrosis.

Collagen depositions in liver tissue were detected by Masson staining, and blue stain indicated collagen fibrils and red stain indicated muscle fiber. In CCl4 treated group (Fig. 3B), massive collagen depositions were detected in perportal areas and hepatic sinusoids. The expressions of collagen depositions were significantly ameliorated in low and high doses of AA groups (Fig. 3C, D), and no collagen depositions in normal group (Fig. 3A).

In CCl4 treated group, α-SMA-positive staining presented a significantly stronger intensity in the perportal sinusoid and around the bile ductules (Fig. 3F). The expression of α-SMA-positive staining were significantly decreased in low and high doses of AA groups (Fig. 3G, H), and no α-SMA-positive staining in normal group (Fig. 3E). It seems likely that AA administration reduced serum aminotransferase and TNF-α levels caused by CCl4 via hepatocyte necrosis and inflammation, which is further confirmed by the reduced amount of hepatocyte damage, collagen deposition and α-SMA expression.

3.3. Effects of acanthoic acid on hepatic α-SMA, Collagen-I, MMP-13 and TIMP-1 expressions

The effects of AA on hepatic fibrotic markers, such as α-SMA and collagen I, were analyzed. CCl4 administration significantly upregulated the protein expression of hepatic α-SMA compared
with normal group, while AA significantly attenuated protein expression of α-SMA. Liver fibrosis accompanies with producing type I collagen molecules. Thus, the effects of AA on CCl₄-induced collagen I accumulation was then analyzed. As expected, the levels of hepatic collagen I protein were obviously increased in mice administered with CCl₄. And AA significantly attenuated CCl₄-induced elevation of hepatic collagen I protein (Fig. 4A).

Degradation of ECM mainly depends on MMP-13, which can be inhibited by TIMP-1. Therefore, the effects of AA on CCl₄-induced expression of hepatic MMP-13 and TIMP-1 were analyzed. As shown in Fig. 4B, CCl₄ administration significantly upregulated the protein expression of hepatic MMP-13. While AA significantly attenuated upregulation of hepatic MMP-13 protein induced by CCl₄. The levels of hepatic TIMP-1 protein were significantly increased in mice administered with CCl₄. And AA significantly attenuated CCl₄-induced upregulation of hepatic TIMP-1 protein (Fig. 4B). The results indicated that AA could regulate the ratio of MMP-13/TIMP-1 to improve the balance of ECM, further reverse the development of liver fibrosis.

3.4. Effects of acanthoic acid on LXRα and NF-κB/İkB-α expressions

Since LXRs show an unexpected role for LXRs signaling in the modulation of hepatic stellate cell function, the effects of AA on LXRα and LXRβ were detected in CCl₄-treated mice. With CCl₄ treatment, LXRα and LXRβ presented different expressions in mice liver. The expression of LXRα showed a little alternation between CCl₄ group and normal group, and the expression of LXRβ was significantly decreased in CCl₄ group compared with that in normal group (Fig. 5A). However, it is interesting that AA administration resulted in upregulating both LXRα and LXRβ.

Based on regulation of inflammation, the study explored downstream signaling that activation of LXRs antagonized inflammatory gene expression, such as NF-κB. Underlying CCl₄-treatment, the IkB-α protein level significantly declined in CCl₄-treated mice liver compared with normal group. Following IkB-α degradation, NF-κB was released from the physical restriction imposed by IkB-α and translocated to the nucleus. Upon AA administration, nuclear translocation of NF-κB still occurred in CCl₄-treated mice, but was less significant (Fig. 5B). Simultaneously, the expression of IkB-α protein was also enhanced by AA administration compared with that in CCl₄-treated group. Therefore, the results indicated that AA might activate LXRα and LXRβ, and follow with inhibit NF-κB translocation to modulate liver fibrosis caused by CCl₄-treatment. The results also suggested that LXRs may orchestrate cross-talk between inflammatory and fibrogenic pathways in CCl₄-treated mice.

3.5. Influence of acanthoic acid on LXRα protein in TGF-β HSC-T6 cells

The results in vivo demonstrated that AA might ameliorate liver fibrosis induced by CCl₄ via activating of LXRα and LXRβ. However, hepatic stellate cells play an important role in the development of liver fibrosis. The major event in hepatic fibrogenesis is the transdifferentiation of quiescent HSCs to a myofibroblastic cell type. Among the secreted cytokines, TGF-β plays a pivotal role in the initiation, promotion, and progression of HSC activation, resulting in increased synthesis of ECM components [21]. And we selected TGF-β (5 ng/ml) to induce the activation of HSC-T6 according the reference [21].

Firstly, we detected the cytotoxicity of AA on HSC-T6 cell by MTT method. Our results showed that AA with a dosage from 0.78 to 50 μmol/l presented almost very low cytotoxicity on HSC-T6 within 24 h (Fig. 6A). Therefore, we chose concentrations of 1, 3, and 10 μmol/l for further analysis.

Activated HSC-T6 cells were stimulated with TGF-β for 2 h before AA administration, and then followed with indicated concentration of AA for next 24 h. As shown in Fig. 6B, C and D, AA (3 and 10 μmol/l) significantly inhibited the increasing α-SMA expression stimulated by TGF-β, and increased expression of LXRβ.
However, AA did not significantly increase the expression of LXRα, which was different with that in vivo.

4. Discussion

Liver fibrosis is characterized by excessive ECM and release of inflammatory cytokines with activation of HSCs. CCl₄-induced liver fibrosis is a well-established animal liver fibrosis model of chronic liver injury. The symptoms of CCl₄ model are similar to those of chronic liver injury in humans [22]. _A. koreanum_ Nakai is widely used in Asia as food or herbal remedies on anti-fatigue, anti-hypoxia, increasing energy and improving circulation activities, and also is used in the therapy of hepatitis and cancer [14]. Acanthoic acid is the major constituent of _A. koreanum_ Nakai that showed strong hepatic protective effect against APAP or D-GalN/
LPS induced liver injury in mice. In this study, we investigated whether AA ameliorated the development of liver fibrosis caused by CCl₄ via activation of LXRs signal. And the AA dose were 20 or 50 mg/kg for mice in the study according our previous research and the LD₅₀ of AA (300 mg/kg) provided by Clearsynth Labs Pvt. Ltd. (India). Also based on the formula for dose translation based on BSA [23], 50 mg/kg for mice would be 4.05 mg/kg for human, which also might be eligible for preclinical study.

CCl₄ is metabolized by cytochrome P450 and produced trichloromethyl free radical, which suppress calcium pump activities on cellular membrane and mitochondrial membrane [24]. Thus, it would induce hepatocyte necrosis and cause intracytoplasm aminotransferase infiltrate into blood with high serum ALT and AST levels [25,26]. Increasing evidence demonstrates that activation of HSCs has involved a variety of pro-inflammatory and pro-fibrogenic factors. TNF-α, an inflammatory cytokine, plays a pro-fibrogenic function by activating HSCs in the process of hepatic fibrosis [27]. We found that serum ALT, AST and TNF-α levels were increased in mice treated with CCl₄, and significantly reduced by AA administration, which were consistent with previous study.
expression strategies. Our result is similar with their conclusion, activated nuclear receptors, based on different cells and gene and metabolism. Their study has also indicated that acanthoic acid numerous biological processes such as reproduction, development, and liver fibrogenesis in CCl₄ [28]. Indeed, the present results showed that AA ameliorated CCl₄-induced inflammation and subsequent inflammatory response through inhibiting TNF-α level.

It is widely accepted that the major cellular event in the development and progression of hepatic fibrosis is the activation of HSCs [29]. Up-regulating of liver fibrosis markers, such as α-SMA and collagen I, indicated the initiation of HSCs activation and liver fibrogenesis in CCl₄-treated mice. Indeed, the present study showed that α-SMA and collagen I were significantly decreased by AA administration. These results suggested that AA inhibited HSCs activation during the pathogenesis of CCl₄-induced hepatic fibrosis. Disequilibrium of MMPs and TIMPs is the foundation for ECM disorder, which leads to excessive deposition of ECM and the process of fibrogenesis. In general, the balanced between synthesis and degradation of ECM could maintain the function and phenotype of quiescent hepatic stellate cells [30]. The results indicated that AA could regulate the ratio of MMP-13/TIMP-1 to improve the balance of ECM, further reverse the development of liver fibrosis. Histological and immunohistochemical staining also supported the current results that AA showed antifibrogenic potential in CCl₄-induced mice. The similar results were also found in vitro study.

Previous study has indicated that certain acanthoic acid diterpenes would efficiently activate LXRs and exert beneficial effects from a cardiovascular standpoint via LXR-dependent mechanisms [31]. LXRs regulate the expression of genes involved in numerous biological processes such as reproduction, development, and metabolism. Their study has also indicated that acanthoic acid exerted anti-inflammatory actions in various animal models and activated nuclear receptors, based on different cells and gene expression strategies. Our result is similar with their conclusion, which demonstrated that AA could activate the expressions of LXRα and LXRβ to modulate liver fibrosis induced by CCl₄. In addition to modulating cholesterol homeostasis, LXRs have emerged as important regulators of inflammatory gene expression and mediated anti-proliferative effects in T cells during the innate immunity [32,33]. In macrophages, LXRs also present a negative regulator of inflammatory. Once activated by inflammatory stimulus, LXRs signal could inhibit the production of inflammatory mediators and modulate immune responses. Analysis of the pattern of nuclear receptor expression in myofibroblastic stellate cells indicated that LXRα and RXRβ are predominant, contrasting with a faint expression of LXRβ. LXRs also downregulate the fibrogenic properties of myofibroblastic stellate cells, as reflected by reduced expression of collagen I and proinflammatory genes. Activation of LXRs also induces stellate cell features reminiscent of a reversion from the fibrogenic/myofibroblastic to a quiescent phenotype, including loss in smooth muscle alpha actin expression and parallel induction of SREBP-1 c and accumulation of neutral lipids. Considering the inflammation throughout liver fibrosis, the role of LXRs in liver fibrosis might be related with anti-inflammation. LXR ligands attenuate inflammation in mouse models of atherosclerosis and limit the proliferation of antigen-stimulated T cells. Therefore, AA might ameliorate liver fibrosis via activation of LXRs for its intervention of inflammation.

In the present study, we focused on the inhibition of liver fibrosis, HSCs activation, and the possible molecular mechanisms. Especially AA could modulate liver fibrosis via activation of LXRα and LXRβ, but inhibit HSCs activation only via activation of LXRβ. Interestingly, AA do not seem to influence LXRα in vitro as that in vivo, indicating that antifibrotic mechanism of AA via LXRβ. Both CCl₄-treated mice and TGF-β-stimulated HSCs studies have been established to reveal the role of LXRβ signaling in modulating liver fibrosis and inflammation. In conclusion, our results suggest that AA might ameliorate the liver fibrosis induced by carbon tetrachloride in mice via activating of LXRα and LXRβ, and inhibit activated HSCs via activating of LXRβ. The present study provides compelling evidence for the prevention and treatment of liver fibrosis.
Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

Acknowledgment

This study was supported by a grant from the National Natural Science Foundation of China, Nos. 81160538 (Ji-Xing Nan) and 81260497 (Yan-Ling Wu). Also this study was supported by the Research Fund for the Doctoral Program of Higher Education (20122201110001) and Science and Technology Department of Jilin Province (20130206052YY) of Ji-Xing Nan.

References