Original Article
Obeticholic acid improves hepatic steatosis and inflammation by inhibiting NLRP3 inflammasome activation

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Abstract: Background and Aim: Several pre-clinical and clinical researches have proved that obeticholic acid (OCA) has a potential therapeutic effect on non-alcoholic steatohepatitis (NASH). Our aim was to investigate whether the therapeutic effect of OCA on NASH was attributed to its inhibition effect on cytosolic sensor NLR family pyrin domain containing 3 (NLRP3) inflammasome activation. Methods: We used mice model of methionine-choline-deficient (MCD) diet induced NASH. At different fibrosis stages, the expressions of NLRP3, caspase-1 and IL-1β were analyzed by means of immunohistochemistry and western blot respectively. After daily gavage of 0.4 mg of OCA or vehicle for 24 days, we evaluated the direct effect of OCA on NLRP3 inflammasome activation by analyzing the expressions of NLRP3 and IL-1β. Additionally, liver function and liver histology of mice were assessed. The expressions of NLRP3 and IL-1β above and the expressions of fibrosis-related genes were analyzed by quantitative real-time polymerase chain reaction (PCR). Results: NLRP3 inflammasome activation could be observed in liver fibrosis, and we found that the expressions of NLRP3, caspase-1 and IL-1β gradually increased to peak at stage 2-3 but decreased significantly at stage 4 of liver fibrosis in MCD mice model. We also found that short-term OCA treatment could significantly down-regulate the expressions of NLRP3 and IL-1β and therefore improved NASH-associated steatosis and inflammation. Conclusions: NLRP3 inflammasome could be activated and might have an essential role in NASH progression, and short-term OCA treatment could have a potential therapeutic effect on NASH-associated steatosis and inflammation by inhibiting NLRP3 inflammasome activation.

Keywords: Obeticholic acid, NLRP3 inflammasome, mice model, non-alcoholic steatohepatitis

Introduction
Nonalcoholic fatty liver disease (NAFLD) has a prevalence rate of more than 30% in Western countries, and is becoming one of the most common chronic liver diseases worldwide [1, 2]. The spectrum of NAFLD includes simple steatosis, steatohepatitis (NASH) with or without fibrosis, cirrhosis, and hepatocellular carcinoma [1]. Approximately 20% of NAFLD may progress to develop chronic hepatic inflammation (NASH) [3, 4]. Recently, many researchers have focus on the investigation of NAFLD and have tried to clarify the mechanism of this disease. The “two-hit” hypothesis which was proposed in 1998 has explained some of the general mechanisms [5]. But, to date, the detailed mechanisms of NAFLD still remain unclear and need to be elucidated.

Inflammasome activation has been recently recognized to play an important role in the development of many liver diseases, such as alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) [6-9]. Inflammasomes are cytoplasmic multi-protein complexes composed of one of several NLR and PYHIN proteins, including NLRP1, NLRP3, NLRC4, and AIM2. Inflammasomes could response to multiple endogenous or exogenous pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), and then be activated to induce maturation of effector pro-inflammatory cytokines such as pro-IL-
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1β and pro-IL-18 [10, 11]. The NLRP3 inflammasome, one of the inflammasomes family members, has been widely concerned and studied nowadays.

NLRP3 inflammasome is a large intracellular multi-protein complex, which consists of NLRP3, adaptor proteins such as the apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC), and the serine protease caspase 1 (Casp1) [12]. A variety of activators, including crystals, large particles such as silica, asbestos, urate, and ATP via the P2X7 receptor, and reactive oxygen species, can induce activation of NLRP3 inflammasome and result in the secretion of effector IL-1β and IL-18 [12, 13]. NLRP3 inflammasome activation has been proved to be necessary for hepatic inflammation and fibrosis [7, 12, 14, 15]. The mechanisms of NLRP3 inflammasome activation induced liver fibrosis have been reported to be associated with the inflammasome activation in HSCs and pro-inflammatory IL-1β signaling pathway [16, 17]. However, the mechanisms and extents of NLRP3 inflammasome activation at different stages of NASH with liver fibrosis remain poorly understood.

Non-alcoholic fatty liver disease (NAFLD) has a prevalence of 20-30% in the general population and up to 75-100% in obese individuals [18, 19]. Despite its high prevalence, factors leading to progression from NAFLD to NASH remain poorly understood and there are no approved therapies. So far, Vitamin E and thiazolidinediones have been studied for the treatment of non-alcoholic steatohepatitis [20, 21], but the long-term efficacy and safety of these drugs remain uncertain. OCA, a 6a-ethyl derivative of chenodeoxycholic acid, is another drug under clinical research for NASH therapy [22, 23].

OCA, a synthetic variant of the natural bile acid chenodeoxycholic acid, is a potent activator of farnesoid X nuclear receptor. Farnesoid X nuclear receptor, which is one of the nuclear receptor superfamilies, is mainly expressed in liver, intestine, and kidney. It regulates a wide variety of target genes critically involved in the control of bile acid synthesis and transport, lipid metabolism, and glucose homeostasis [24]. OCA, which is a first-in-class selective FXR agonist, has been approved for primary biliary cirrhosis (PBC) therapy since last year, and also been proven having anti-inflammatory and anti-fibrotic effects in the liver by a range of preclinical studies [25, 26]. However, the specific mechanisms of OCA on the improvement of liver inflammation and fibrosis remain unclear.

Our study was to investigate the condition of NLRP3 inflammasome activation in liver fibrosis, and to investigate whether the therapeutic effect of OCA on NASH was attributed to its inhibition effect on NLRP3 inflammasome activation.

Materials and methods

Animals

All C57BL/6 mice were supplied by Beijing Vital River Laboratory Animal Technology Co. Ltd, China. They were kept at 25°C with a 12 h light/dark cycle, and allowed standard chow and water ad libitum until the time of the study. All of this study’s protocols were approved by the Animal Experimental Ethical Committee of Peking University People’s Hospital (No.2015-21).

NASH model for liver fibrosis

6-8 week-old male C57BL/6 mice were fed with a methionine-choline-deficient (MCD) diet (ICN Biochemicals, San Diego, CA) for 24 weeks to induce histological features of NASH, and then these mice were sacrificed at indicated time (0, 4, 6, 8, 12, 18, 20 and 24 weeks) to obtain specimens of different fibrosis stages (n = 7 per group). Mice had unrestricted access to food and water.

NASH model for OCA treatment

6-8 week-old male C57BL/6 mice were fed a MCD diet (ICN Biochemicals, San Diego, CA) or standard chow for 6 weeks, and then these mice were administrated with OCA once daily at a dose of 0.4 mg or drug vehicle for 24 days (n =5 for each vehicle group, n = 8 for OCA group). OCA was supplied by Kawin Technology Co. Ltd, China. Methylcellulose was purchased from Sigma-Aldrich, St. Louis, MO, USA, and used as drug vehicle.

Biochemical analysis

Blood samples were centrifuged and serum samples were collected. Aspartate aminotrans-
ferase (AST), alanine aminotransferase (ALT), high density lipoprotein (HDL-c), low density lipoprotein (LDL-c), cholesterol (CHO), triglycer-ide (TG), and uric acid (UA) were measured by the 7180 automatic analyzer (Hitachi, Tokyo, Japan).

**Hydroxyproline (HYP) detection**

The extent of the liver fibrosis was also determined by estimating the total liver collagen content, as reflected by measurements of the HYP content in the liver. HYP content was measured using a test kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

**Histology**

All mice were anesthetized and liver tissues were collected at the time of sacrifice. Liver specimens from the left lateral lobe of each animal were formalin-fixed, paraffin-embedded and sectioned for hematoxylin and eosin (H&E), Sirius Red staining, or immunohistochemistry for histological analysis. NAFLD activity scores of steatosis and inflammation were semiquantitatively evaluated by pathologists using the scoring system of NAFLD proposed in 2005 [27]. Liver fibrosis was assessed using Sirius Red, and fibrosis stages ranged from 0 to 4 with 0 being within normal limits and 4 being most severe [27]. The most severe areas of hepatic fibrosis of representative histology sections were photographed using an Olimpus microscope.

**Immunohistochemistry**

Immunohistochemistry staining for NLRP3 (Rabbit polyclonal to NLRP3, Abcam, Cambrigde, MA, USA) was performed in formalin-fixed, paraffin-embedded liver sections according to the manufacturer’s instruction (Abcam, Cambrigde, MA, USA). The antigen was repaired with sodium citrate buffer solution (PH 6.0) heating for 20 minutes at 95°C. The primary antibody of NLRP3 was used in a dilution of 1:500. Digital light microscopic images were recorded and analyzed with Image-pro plus 6.0.

**Quantitative real-time PCR**

Total RNA was isolated from liver with TRI-ZOL reagent (Invitrogen, Life Technologies, Carlsbad, USA). 1 μg of RNA was used for generate cDNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Waltham, MA, USA). Gene expression was quantified by real-time PCR using the Roche Light-Cycler® 480II Sequence Detection system (Roche Applied Science, Basel, Switzerland). Gene expression was normalized by β-actin expression.

**Western blot**

Whole cell lysates were extracted from liver tissues. Samples with equal amounts of protein were separated in polyacrylamide gel, transferred and identified on nitrocellulose membrane with caspase-1 antibody (Abcam, Cambrigde, MA, USA) and IL-1β antibody (Santa Cruz Bio-technology Inc., Dallas, TX, USA) followed by HRP-labelled secondary antibodies. The primary antibodies of caspase-1 and IL-1β were both used in a dilution of 1:500. Beta actin (Abcam, Cambrigde, MA, USA) was used as loading control. Image J was used to analyze gray values of results.

**Statistical analysis**

Analyses were performed with Graph Pad Prism 6.0 (Graph Pad Software Inc., La Jolla, CA, USA). Data are expressed as mean ± SEM. Differences were analyzed by Student’s t test. p value <0.05 was considered significant.

**Results**

The expressions of NLRP3, caspase-1 and IL-1β were different at different fibrosis stages in MCD mice model of NASH, which increased to peak at stage 2-3 but decreased dramatically at stage 4

C57BL/6 mice were fed with MCD diet at 8 weeks of age to induce NASH, and then these mice were sacrificed at different indicated time to acquire different stages of NASH, which were divided by stages of liver fibrosis in our study. Stages of liver fibrosis of MCD mice were determined and divided into 4 stages by Sirius Red staining (Figure 1A). In our mice model, we found that the contents of L-HYP were gradually increasing across the liver fibrosis stages (Figure 1B).

Histologically, we found that the expression of NLRP3 was upregulated across liver fibrosis
Figure 1. L-HYP was elevated across progression of NASH with liver fibrosis in MCD mice model. Wild-type C57BL/6 mice were fed with methionine-choline-deficient (MCD) diet and sacrificed at different indicated time to acquire NASH with different stages of liver fibrosis. A: Sirius Red staining for determining stages of liver fibrosis (magnification × 100). B: L-HYP expression at different stages of liver fibrosis. Values are Mean ± SEM, n = 7 animals per group. L-HYP: L-hydroxyproline.

Figure 2. The expression of NLRP3 was upregulated in liver fibrosis stages of NASH and increased to peak at stage 3, but decreased significantly at stage 4. The expression of NLRP3 was observed at different fibrosis stages of NASH. A: Histologically, we detected the expression of NLRP3 at different liver fibrosis stages by Immunohistochemistry (magnification × 200). B: The expression of NLRP3 was analyzed using Image-pro Plus 6.0. Values are Mean ± SEM, n = 7 animals per group. *P<0.05, **P<0.01, ***P<0.001. NLRP3: NOD-like receptor protein 3; IL-1β: Interleukine-1 beta; IOD: Integrated option density.

The expression of NLRP3 was upregulated in liver fibrosis stages of NASH and increased to peak at stage 3, but decreased significantly at stage 4. The expression of NLRP3 was observed at different fibrosis stages of NASH. A: Histologically, we detected the expression of NLRP3 at different liver fibrosis stages by Immunohistochemistry (magnification × 200). B: The expression of NLRP3 was analyzed using Image-pro Plus 6.0. Values are Mean ± SEM, n = 7 animals per group. *P<0.05, **P<0.01, ***P<0.001. NLRP3: NOD-like receptor protein 3; IL-1β: Interleukine-1 beta; IOD: Integrated option density.

stages of NASH compared to stage 0, and the expression level gradually increased to peak at stage 3 but decreased dramatically at stage 4 of liver fibrosis in MCD mice model (Figure 2A, 2B). To further test whether the NLRP3 inflammasome was activated in MCD mice model of
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NASH, we next investigated the expressions of caspase-1 and IL-1β, which were the effector molecules of NLRP3 inflammasome (Figure 3A-C). As was showed in Figure 3A-C, the expressions of caspase-1 and IL-1β increased to peak at stage 2 of liver fibrosis and remained high level at stage 3, but the expressions of both proteins decreased significantly at stage 4.

Short-term OCA treatment down-regulated the expressions of NLRP3 and IL-1β and lowered the concentration of blood UA in MCD mice model of NASH

OCA is now a drug under clinical research for NASH therapy and has been proven to have some favorable effects, but its mechanism of action remains unclear. In our study, we fed wild-type C57BL/6 mice with MCD diet or standard chow at 6-8 weeks of age for 6 weeks, and then administrated them with OCA once daily at a dose of 0.4 mg or vehicle for 24 days. During drug treatment, there was one mouse dead in OCA treatment group. In order to investigate whether OCA treatment could regulate NLRP3 inflammasome activation, we analyzed the mRNA expression levels of NLRP3 and IL-1β in liver tissues by means of real-time PCR. In line with our finding above, the mRNA expression levels of NLRP3 and IL-1β were significantly higher in liver samples of mice fed with MCD diet compared to mice fed with standard chow (NLRP3: \( P < 0.001 \); IL-1β: \( P < 0.05 \)). Notably, aforementioned mRNA expression levels were significantly reduced in mice treated with OCA for 24 days when compared to mice treated with vehicle (NLRP3: \( P < 0.05 \); IL-1β: \( P < 0.05 \)) (Figure 4A, 4B).

Blood UA is one of the agonists which could induce NLRP3 inflammasome activation and IL-1β production [28-30]. So, we next detected the blood UA concentration of all groups. As was showed in Figure 4C, after administrated with vehicle for 24 days, mice fed with MCD diet had a significantly higher concentration of blood UA compared to mice on standard chow (\( P < 0.001 \)). Notably, mice with OCA treatment for 24 days showed a significantly lower concentration of blood UA when compared to diet-matched mice treated with vehicle (\( P < 0.01 \)).
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Figure 4. Short-term OCA treatment down-regulated the mRNA expressions of NLRP3 and IL-1β and decreased the blood UA concentration in MCD mice model. After 24 days of drug treatment, we analyzed the liver mRNA expressions of NLRP3 and IL-1β and detected the blood UA concentration of three mice groups. A: The mRNA expression level of NLRP3 in three groups was detected. B: The mRNA expression level of IL-1β in three groups was detected. C: The blood UA concentration of three groups was detected. Values are Mean ± SEM, n = 5 animals for each vehicle group, n = 7 animals for OCA group. *P<0.05, **P<0.01, ***P<0.001. OCA: obeticholic acid; NLRP3: NOD-like receptor protein 3; IL-1β: Interleukine-1 beta; UA: uric acid.

Figure 5. Histologically, short-term OCA treatment improved hepatic steatosis and inflammation by inhibiting NLRP3 inflammasome activation above in MCD mice model. Wild-type C57BL/6 mice were fed with MCD diet or standard chow at 8 weeks of age for 6 weeks, and then were administrated with OCA once daily at a dose of 0.4 mg or vehicle for 24 days. After 24 days of drug treatment, we observed liver histology (A, H&E stain) and evaluated the NAFLD activity score (B) of three mice groups. Values are Mean ± SEM, n = 5 animals for each vehicle group, n = 7 animals for OCA group. *P<0.05, **P<0.01. OCA: obeticholic acid. Magnification × 100 (left, B); Magnification × 200 (right, B).
Short-term OCA treatment improved liver steatosis and inflammation by inhibiting NLRP3 inflammasome activation above in MCD mice model of NASH

By feeding mice with MCD diet for 9 weeks, we acquired mice of NASH with fibrosis ranging from stage 1 to 3. According to our finding above, NLRP3 inflammasome was active across these liver fibrosis stages and could be suppressed by 24-days’ OCA treatment. In order to investigate whether NLRP3 inflammasome inhibition by short-term OCA treatment could improve NASH-associated steatosis and inflammation, we evaluated the liver histologic changes and detected the blood ALT and lipid indexes after drug treatment. As was showed in Figure 5A, 5B, liver histology and NAFLD activity score revealed that MCD diet significantly induced liver steatosis and inflammation in mice compared to mice fed with standard chow (Steatosis: P<0.01; Inflammation: P<0.01), and that OCA treatment significantly alleviated liver steatosis and inflammation in mice compared to vehicle treatment group fed with MCD diet (Steatosis: P<0.05; Inflammation: P<0.05).

Biochemical analysis revealed that blood ALT and AST levels were significantly increased in mice fed with MCD diet compared to mice on standard chow (P<0.001), and that OCA treatment significantly decreased ALT and AST levels in mice compared to diet-matched mice treated with vehicle (ALT: P<0.01; AST: P<0.001) (Figure 6A, 6B). Except for ALT and AST, we simultaneously tested the blood lipid indexes of our mice model, which included LDL, HDL, CHO, and TG. As we showed in Figure 6C-F, all of the lipid indexes were lower in mice fed with MCD diet than that in mice fed with standard chow (LDL: not significantly; HDL: P<0.001; CHO: P<0.001; TG: not significantly). When compared to mice treated with vehicle, diet-matched mice treated with OCA also had decreased levels of their blood lipid indexes (LDL: not significantly; HDL: P<0.05; CHO: P<0.05; TG: P<0.01).

Short-term OCA treatment did not attenuate fibrosis in liver histology but improved fibrogenesis in MCD mice model of NASH

In our study, we analyzed liver fibrosis in different mice groups after drug treatment by Sirius Red staining and found that MCD diet significantly induced liver fibrosis of NASH compared to chow diet (P<0.001) and that OCA treatment for 24 days did not significantly attenuate fibrosis in liver histology compared to diet-matched mice treated with vehicle (Figure 7A, 7B).

But we found that the protein expression level of L-HYP and the mRNA expression levels of α-SMA and COL1A1 in liver tissues, all of which were associated with fibrogenesis, increased significantly in mice fed with MCD diet compared to mice on standard chow (L-HYP: P<0.01; α-SMA: P<0.01; COL1A1: P<0.001), but decreased significantly in mice with OCA treatment for 24 days when compared to diet-matched mice treated with vehicle (L-HYP: P<0.05; α-SMA: P<0.05; COL1A1: P<0.05) (Figure 7C-E).

Discussion

NLRP3 inflammasome activation has been proved to be necessary for hepatic inflammation and fibrosis, and play a key role in the development from NAFLD to NASH [7, 12, 14, 15, 31]. The mechanisms of NLRP3 inflammasome activation induced liver fibrosis have been reported to be associated with the inflammasome activation in Hepatic stellate cells (HSCs) and pro-inflammatory IL-1β signaling pathway [16, 17]. However, the mechanisms and extents of NLRP3 inflammasome activation at different stages of liver fibrosis remain poorly understood.

Our study found that the expressions of NLRP3, caspase-1 and IL-1β were all upregulated in NASH-associated liver fibrosis, which proved that NLRP3 inflammasome was activated in these NASH mice models. Our findings above were consistent with other researchers’ observations [9, 32, 33]. Most noteworthy, we investigated NLRP3 inflammasome activation at different stages of liver fibrosis of NASH, and found that the expression levels of NLRP3 gradually increased to peak at stage 3 but decreased dramatically at stage 4 of liver fibrosis of NASH, and that the expression level of caspase-1 and IL-1β increased to peak at stage 2 and remained high level at stage 3 but decreased significantly at stage 4 of liver fibrosis in MCD mice model. Our results indicated that activation of NLRP3 inflammasome reached the highest level at stages 2-3 of liver fibrosis of NASH and had a significant decrease...
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Figure 6. Biochemical analysis revealed that short-term OCA treatment decreased the blood levels of ALT, AST and the lipid indexes in MCD mice model. A-F: After 24 days of drug treatment, we detected the blood ALT and AST of three mice groups, and we also detected the blood LDL, HDL, CHO, and TG. Values are Mean ± SEM, n = 5 animals for each vehicle group, n = 7 animals for OCA group. *P<0.05, **P<0.01, ***P<0.001. OCA: obeticholic acid; ALT: alanine aminotransferase; AST: Aspartate aminotransferase; LDL: low density lipoprotein; HDL: high density lipoprotein; CHO: cholesterol; TG: triglyceride.

Figure 7. Short-term OCA treatment did not significantly attenuate liver fibrosis but reduced NASH-associated fibrogenesis dramatically in MCD mice model. At end of study, we analyzed liver fibrosis by Sirius Red staining and detected the protein expression level of L-HYP and the mRNA expression levels of α-SMA and COL1A1 in liver tissues of three mice groups. A. Sirius Red staining showed liver fibrosis of three mice groups (magnification × 100). B. Liver fibrosis stages of three mice groups were assessed. C. The protein expression level of L-HYP in liver tissues of three mice groups was detected. D-E. The mRNA expression levels of α-SMA and COL1A1 in liver tissues of three mice groups were detected. Values are Mean ± SEM, n = 5 animals for each vehicle group, n = 7 animals for OCA group. *P<0.05, **P<0.01, ***P<0.001. OCA: obeticholic acid; L-HYP: L-hydroxyproline; α-SMA: alpha smooth muscle actin; COL1A1: Collagen Type I Alpha 1.
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at stage 4. We speculated that these changes above might be partially attributed to the substantial death of hepatic cells in late fibrosis stages, which led to reductions of NLRP3 inflammasome. One of the limitations of our study was that we did not inhibit NLRP3 inflammasome activation by using caspase-1 inhibitors or suppress IL-1β signal pathway by using IL-1β antagonists.

Despite that NAFLD has a high prevalence, the detailed mechanism of the progression from NAFLD to NASH has not been completely explained so far, and, therefore, no effective treatment has been developed. So far, Vitamin E and thiazolidinediones have been studied for the treatment of non-alcoholic steatohepatitis [20], but the long-term efficacy and safety of these drugs remain uncertain. OCA, a 6a-ethyl derivative of chenodeoxycholic acid, is another drug under clinical research for NASH therapy [22, 23]. In pre-clinical studies, OCA improved hepatic steatosis, fibrosis, and portal hypertension [25, 26]. In clinical studies, OCA improved insulin sensitivity and reduced serum alanine aminotransferase concentrations, and also improved NASH-associated steatosis, inflammation, and fibrosis [22, 23].

Our finding indicated that MCD diet induced liver steatosis and inflammation in mice, which mimicked the histological lesions of human NASH, and that short-term OCA treatment improved NASH-associated pathological manifestations above in MCD mice model, which was consistent with previous studies above. But it was noteworthy that short-term OCA treatment could inhibit NLRP3 inflammasome activation by down-regulating the expressions of NLRP3 and IL-1β. This finding perhaps indicated that activation of farnesoid X nuclear receptor could interfere with NLRP3 inflammasome activation by regulating the expressions of components of NLRP3 inflammasome, or by regulating the blood uric acid concentration which is one of the activators of NLRP3 inflammasome. Guo et al. demonstrated that bile acids could inhibit NLRP3 inflammasome activation via TGR5-cAMP-PKA axis, in which PKA kinase induced NLRP3 phosphorylation on Ser 291 and the ubiquitination of NLRP3 [34]. As was one kind of bile acids, OCA might also inhibit NLRP3 inflammasome activation via TGR5-cAMP-PKA axis. So, the therapeutic effect of OCA on NASH-associated liver steatosis and inflammation was likely attributed to its inhibiting effect on NLRP3 inflammasome activation which had been proved to be necessary for hepatic inflammation. We also examined whether short-term OCA treatment could improve NASH-associated liver fibrosis. As was showed in our results, short-term OCA treatment did not significantly attenuate liver fibrosis of NASH compared to vehicle treatment in liver histology. But our further exploration indicated that short-term OCA treatment improved liver fibrogenesis by down-regulating the protein expression level of L-HYP and the mRNA expression levels of α-SMA and COL1A1 in liver tissues. So, we speculated that short-term OCA treatment might not have obvious therapeutic effect on liver fibrosis because of short duration of drug treatment, which could be improved by prolonged drug action time.

Our study investigated the effect of NLRP3 inflammasome activation on different liver fibrosis stages of NASH, and further proved the therapeutic effect of OCA on NASH-associated liver steatosis and inflammation. And we also elucidated the possible mechanisms of the therapeutic effect of OCA on NASH by inhibiting NLRP3 inflammasome activation. Importantly, the improvements with OCA treatment confirm that farnesoid X nuclear receptor signalling affects lipid metabolism in the liver, but treatment causes changes in the serum LDL and HDL concentrations that could signal an increased risk of atherogenesis. Thus, short-term OCA therapy improves steatosis and inflammation of NASH, but its long-term safety requires further clarification.

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Disclosure of conflict of interest

None.

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