Chinese Herbal Medicines 11 (2019) 292–298

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Original Article

Berberine reverses LPS-induced repression of CYP7A1 through an anti-inflammatory effect

Bei Cao[a], Run-bin Sun[b], Geng Yan[a], Guang-yu Yang[a], Ji-ye Aa[b,*], Juan Li[a,*]

[a] Phase I Clinical Trial Unit, Nanjing Drum Tower Hospital, The Affiliated Hospital of Medical School of Nanjing University, Nanjing 210008, China
[b] Key Lab of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009, China

ARTICLE INFO

Article history:
Received 13 July 2018
Revised 27 October 2018
Accepted 7 November 2018
Available online 24 May 2019

Keywords:
berberine
bile acids
CYP7A1
inflammation
LPS

ABSTRACT

Objective: To evaluate the anti-inflammatory effect of berberine (BBR) on the lipopolysaccharide (LPS)-induced acute phase response and its modulation of the altered bile acid metabolism induced by LPS treatment.

Methods: An acute phase response was induced by intraperitoneal injection of LPS (5 mg/kg, ip) in C57BL/6J mice, and the BBR treatment group was orally administered with BBR (200 mg/ky, ig). The levels of TNFα, IL-1β and IL-6 in the serum were measured using an ELISA kit, and their expression levels in the liver were measured using qRT-PCR. The bile acid pool was measured using a commercial bile acid kit, and the expression levels of enzymes involved in bile acid metabolism were measured by qRT-PCR. The expression levels of CYP7A1, p65 NF-κB and the MAPK signaling pathway was measured using Western blotting.

Results: LPS treatment suppressed the expression of CYP7A1 and CYP8B1, and the total bile acid pool was also reduced. Pretreatment with BBR inhibited the pro-inflammatory biomarkers TNFα and IL-1β in the serum, as well as the expression of TNFα, IL-1β and iNOS mRNA in the liver. BBR treatment did not affect the reduction in the bile acid pool size induced by LPS, but significantly increased the concentration of bile acids in the liver, which was consistent with the upregulated expression of CYP7A1 and CYP8B1. The MAPK signaling pathway was activated by BBR treatment, while the p65 NF-κB signaling pathway was inhibited.

Conclusion: BBR can offer an anti-inflammatory effect and reverse the inhibition of CYP7A1 and CYP8B1 expression caused by LPS treatment, as well as induce the production of bile acids in liver, probably via MAPK signaling; However, treatment with BBR had no effect on the size of total bile acid pool.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common disease in developed countries (up to 30%), and it is becoming more and more common in developing countries. Non-alcoholic steatohepatitis (NASH) is the most extreme form of NAFLD, including steatosis, fibrosis and inflammation (James & Day, 1998). So far, no drug has been approved by the FDA for treating NASH. The phase 3 trial of obeticholic acid (OCA, OCALIVA®, Intercept Pharmaceuticals, Inc.) for the treatment of NASH had been finished and it showed obvious effect for improving liver fibrosis. The pathogenesis of NASH has not been well elucidated. The widely accepted multihit model of NASH invokes lipid accumulation in the liver as the first hit and the following multihits include oxidative stress, adipocytokine induction, mitochondrial dysfunction, inflammation, among others (Alkhouri, Carter-Kent, & Feldstein, 2011; James & Day, 1998; Polyzos, Kountouras, & Zavos, 2009). The inflammatory effect in NASH is mainly induced by the endotoxins (lipopolysaccharide, LPS) produced by gut microbiota, the accumulation of inflammatory bile acids and other inflammatory factors. LPS is a ligand of TLR4 and can induce an acute phase response. It is widely used to establish inflammation models both on animals and cell lines (Chu et al., 2014; Gao et al., 2014; Lou et al., 2011; Tang et al., 2015).

Traditional Chinese medications are widely used for preventing and treating diseases in China. Berberine (BBR) is extracted from the roots of *Coptis chinensis* Franch. and has long been used as an antibiotic to treat diarrhea. Several studies have shown that BBR can change the composition of the gut microbiota (Xie, Gu, Li, Cui, & Zhang, 2011; Zhang et al., 2012) and suppress the pro-inflammatory response in various cells and animal models, mainly by inhibiting the activation of the nuclear factor kappa B (NF-κB) pathway (Fu et al., 2015; Gao et al., 2014), activating mitogen-activated protein kinase (MAPK) (Gao et al., 2014; Jeong

* Corresponding authors.
E-mail addresses: jiyea@cpu.edu.cn (J.-y. Aa), juanli2003@163.com (J. Li).

https://doi.org/10.1016/j.chmed.2019.05.004
1674-6384/

et al., 2009; Mo et al., 2014) and nuclear factor erythroid-2-related factor-2 (Nrf2) pathways (Mo et al., 2014) and binding to the Toll-like receptor 4/myeloid differentiation factor 2 (TLR4/MD-2) receptor (Chu et al., 2014), among others.

Metabolism is the main elimination pathway of cholesterol. Cholesterol can be metabolized to bile acids in the liver, mainly through the classical pathway and an alternative pathway. In the classical pathway, cholesterol is metabolized by cholesterol 7α-hydroxylase (CYP7A1) and sterol 12α-Hydroxylase (CYP8B1) to form cholic acid (CA), while in the alternative pathway, cholesterol 7α-hydroxylase (CYP27A1) and oxysterol 7α-hydroxylase (CYP7B1) metabolize cholesterol to chenodeoxycholic acid (CDCA) (Russell, 2009; Thomas, Pellicciari, Pruzanski, Auwerx, & Schoonjans, 2008). CYP7A1 is the first and rate-limiting enzyme among these processes. CYP7A1 can be regulated by various factors, including liver X receptor (LXR) (Fu, Csanaky, & Klaassen, 2012; Goodwin et al., 2003; Gupta, Pandak, & Hylemon, 2002), farnesoid X receptor (FXR) (Sinal et al., 2000), Rev-erbα (Duez et al., 2008; Ma et al., 2009), among others. LPS treatment, as well as tumor necrosis factor alpha (TNFα) and interleukin-1 beta (IL-1β), can suppress the expression of CYP7A1 in hamsters (Dikopoulos, Weidenbach, Adler, & Schmid, 2003; Feingold, Spady, Pollock, Moser, & Grunfeld, 1996); Furthermore, three months of treatment of 1.25% cholesterol in the diet can induce an inflammatory effect in the liver and increase the mRNA levels of TNFα and IL-1β and reduce the expression levels of CYP7A1 in FVB/NJ mice (Henkel, Anderson, Dewey, Kavesh, & Green, 2011). NF-κB belongs to the category of "rapid-acting" primary transcription factors, and it is a major transcription factor that regulates genes responsible for the immune response. The known inducers of NF-κB activity are highly variable and include reactive oxygen species (ROS), TNFα, IL-1β, bacterial LPS and ionizing radiation (Basu, Rosenzweig, Youmell, & Price, 1998; Chandel, Trzyna, McClintock, & Schumacker, 2000; Fitzgerald et al., 2007; Qin, Wilson, Lee, Zhao, & Benveniste, 2005; Renard et al., 1997). The MAPK signaling pathway plays an important role in inflammation, and it can modulate the transcriptional activity of NF-κB (Saha, Jana, & Pahan, 2007). Furthermore, it has been shown that MAPK signaling pathway plays an important role in the regulation of CYP7A1 (Kong et al., 2012; Xu et al., 2007). Several studies found that BBR could reverse the inflammatory effect induced by LPS and that it can modulate the expression of cholesterol metabolism enzymes to alter the composition of bile acids; However, it is still unknown on which components of the different pathways BBR acts to modulate inflammation and bile acid metabolism. Therefore, we hypothesized that BBR can inhibit the inflammatory reaction induced by LPS and reverse the inhibition of CYP7A1 expression by reducing the TNFα and IL-1β levels, mainly through the NF-κB and MAPK signaling pathways.

2. Materials and methods

2.1. Chemicals and reagents

BBR ($\geq$ 98%) was purchased from Nanjing Zelang Medical Technology Co., Ltd. (Nanjing, China). LPS from *Escherichia coli* 0111:B4 was obtained from Sigma-Aldrich Co., Ltd. (St. Louis, MO). Chloroform was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China), and the isopropanol and ethanol were purchased from Shanghai Titan Scientific Co., Ltd. (Shanghai, China). Distilled water was produced using a Milli-Q Reagent Water System (Millipore, MA, USA).

2.2. Animal treatment

Fifteen C57BL/6J mice (5–6 weeks, male) were provided by Yangzhou University, China. All mice were housed under SPF conditions and fed with tap water *ad libitum*, under a 12-hour light-dark cycle. All of the animal experiments were performed with the approval of the Animal Ethics Committee of China Pharmaceutical University.

The mice were fed for one week before the experiment for acclimation. The mice were divided into three groups, namely, the vehicle group, the LPS group and the LPS+BBR group ($n = 5$). To investigate the effect of BBR on the LPS-induced decreases in CYP7A1 and CYP8B1 expression, BBR was orally administered at a concentration of 200 mg/kg, b.i.d for 7 d, and mice in the LPS and vehicle groups were gavaged with 0.5% CMC-Na. On the morning of the 8th day, normal saline (200 μL/20 g, ip) was administered to the mice in the vehicle group, LPS (5 mg/kg, ip) was administered to the mice in the LPS and LPS+BBR groups, and the mice in the LPS+BBR group additionally received orally administered BBR (200 mg/kg, ig) at the same time. After 4 h, the mice were sacrificed and the blood, liver, and total intestine were collected and stored at −80 °C until analysis.

2.3. Measurement of serum pro-inflammatory factors and liver function

Serum was obtained by centrifuging blood at 8000 r/min for 10 min. The serum was transferred carefully to avoid the LPS and bacterial contamination. The levels of the inflammatory factors TNFα, IL-1β and interleukin-6 (IL-6) in the serum were measured using ELISA kits (Excell Bio, Shanghai, China) following the manufacturer's protocol. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AKP) levels were measured with kits purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China).

2.4. Bile acid pool determination

The bile acid pool was measured by pooling the total bile acids from the liver, gall bladder, small intestine and its contents, as reported previously (Kong et al., 2012). Briefly, 100 mg of liver tissue was homogenized and extracted with 75% EtOH for 1 h and then centrifuged at 12 000 r/min for 10 min. The entire intestine with its contents was also homogenized and extracted with 75% EtOH for 1 h and centrifuged at 4000 r/min for 10 min, and the gallbladder was diluted with 1 mL PBS. The intestine and gallbladder extractions were diluted 10 fold. The total bile acids were measured using the Total Bile Acids Assay Kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). The bile acid pool was calculated and expressed as the total bile acid per 100 g.

2.5. qRT-PCR analysis

Total RNA was isolated using Trizol Reagent (TaKaRa, Dalian, China), according to the manufacturer's instructions, and the mRNA was quantified and diluted in 0.5 μg/μL DEPC water. The diluted mRNA was reverse-transcribed with RT reagent. The mRNA levels were determined by qRT-PCR using SYBR green supermix (Bio-Rad) in a Bio-Rad real-time PCR machine. The expression levels of CYP7A1, CYP27A1, CYP8B1, CYP7B1, TNFα, IL-1β, IL-6 and inducible nitric oxide synthase (iNOS) were measured. The sequences of the primers used were shown in Table 1. The results were normalized to GAPDH expression.

2.6. Western blot

Livers were collected and one piece was used for the Western blot analysis as described previously. Briefly, 100 mg of liver

Table 1
Primer sequences for qRT-PCR analysis.

Genes	Forward primer sequence (5′ – 3′)	Reverse primer sequence (5′ – 3′)
TNFα	TCAGCCTCTTCTCCTTCCTG	TGAAGAGGACCTGGGAGTAG
IL-1β	TGAACTGAAAGCTCTCCACC	CTGATGTACCAGTTGGGGAA
IL-6	GGTACATCCTCGACGGCATCT	GTGCCTCTTTGCTGCTTTCAC
iNOS	CAGCACAGGAAATGTTTCAGC	TAGCCAGCGTACCGGATGA
CYP7A1	GCATCATAGCTCTTTACCCAC	GGTGTTCTGCAGTCCTGTAAT
CYP27A1	CCTTTGGGACTCGCACCA	GCCCTCCTGTCTCATCACTTG
CYP7B1	CAGCTATGTTCTGGGCAATG	TCGGATGATGCTGGAGTATG
CYP8B1	AGTACACATGGACCCCGACATC	GGGTGCCATCCGGGTTGAG

was homogenized in 1 mL RIPA Lysis Buffer (Beyotime, China) supplemented with protease inhibitors (Phenylmethanesulfonyl fluoride, PMSF, Beyotime, China). The protein concentration was measured using the BCA assay (Beyotime, China). The proteins were separated using 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and were then transferred onto a methanol-equilibrated polyvinylidene difluoride (PVDF) membrane (BioRad, CA, USA). The membrane was blocked with 5% milk in Tris-buffered saline Tween-20 (TBST) for 2 h at room temperature and then incubated at 4 °C overnight with anti-CYP7A1 antibody (1:1000 dilution; Boster Biological Technology, Wuhan, China), anti-p65-NFκB antibody (1:2000 dilution), anti-p-ERK antibody (1:2000 dilution), anti-T-ERK antibody (1:2000 dilution), anti-p-JNK antibody (1:2000 dilution), anti-T-JNK antibody (1:2000 dilution), anti-p-p38 MAPK antibody (1:2000 dilution), anti-T-p38 MAPK antibody (1:2000 dilution) or anti-GAPDH antibody (1:2000 dilution; Boster Biological Technology, Wuhan, China), followed by incubation with the secondary antibody (anti-Rabbit) at a dilution of 1:5000 for 2 h. All the antibodies used in this study were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA) unless otherwise specified. The intensities of the immune-reactive bands were analyzed using a ChemiDoc XRS imager (BioRad, CA, USA).

2.7. Statistical Analysis

All data are expressed as the mean ± SD. The differences among the groups were tested by one-way ANOVA followed by Student's *t*-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. BBR showed anti-inflammatory effects in both serum and liver

The serum levels of inflammatory factors, as well as the expression levels of inflammatory factor genes in the liver, were measured after the mice were sacrificed. LPS treatment induced an obvious acute phase response, activated the NF-κB pathway, increased the TNFα, IL-1β and IL-6 levels in the serum (Fig. 1), and significantly increased the TNFα, IL-1β, IL-6 and iNOS mRNA levels in the liver (Fig. 2). BBR treatment could significantly decrease the levels of TNFα (Fig. 1A) and IL-1β (Fig. 1B) in the serum as well as the expression of TNFα (Fig. 2A) and IL-1β (Fig. 2B) in the liver; however, BBR had little effect on the IL-6 level in the serum (Fig. 1C), whereas it decreased the mRNA expression in the liver (Fig. 2C).

3.2. BBR can reverse increase in AKP level induced by LPS

AST, ALT and AKP can indicate the changes in liver function. Four hours after LPS injection (5 mg/kg, ip), the levels of AST and ALT were unchanged; However, the AKP level was significantly increased by about 19.6% ($P = 0.014$), Table 2. Administration of BBR (150 mg/kg, ig, b.i.d) for 7 d did not change the AST and ALT levels significantly, and the decreased AKP level caused by LPS treatment was reversed by BBR treatment.

3.3. BBR can promote production of bile acids in liver

Cholesterol can be metabolized to cholic acid and excreted into the bile. The total bile acid pool was measured to evaluate changes in cholesterol and bile acid metabolism (Fig. 3). The bile acid pool was significantly reduced by LPS treatment (30.9%, $P = 0.016$, Fig. 3D). In the liver and intestine, bile acids were reduced by 38.4% ($P < 0.001$, Fig. 3A) and 38.5% ($P = 0.002$, Fig. 3C), respectively. However, the bile acids were elevated in the gallbladder by 126.3% ($P = 0.002$, Fig. 3B). BBR could promote the expression of bile acids in the liver (78.2%, $P = 0.001$, Fig. 3A), whereas it had no effect on the bile acids in the gallbladder and intestine or the total bile acid pool.

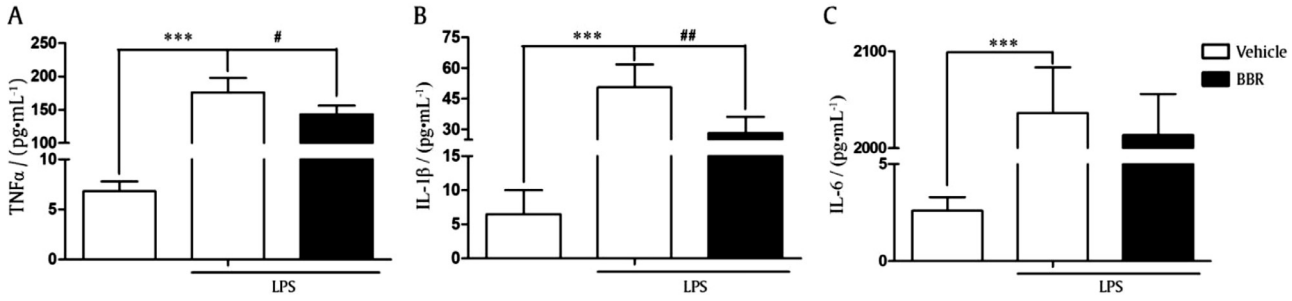

Fig. 1. Effect of berberine on inflammatory factors in serum.
C57BL/6 mice were treated with berberine (150 mg/kg/d, ig, b.i.d) or vehicle (CMC Na) for one week, and on 8th day, the LPS and LPS+BBR groups were injected with LPS (5 mg/kg, ip). Levels of serum inflammatory factors TNFα (A), IL-1β (B) and IL-6 (C) were determined. $^{***}P < 0.001$ *vs* vehicle group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* LPS group.

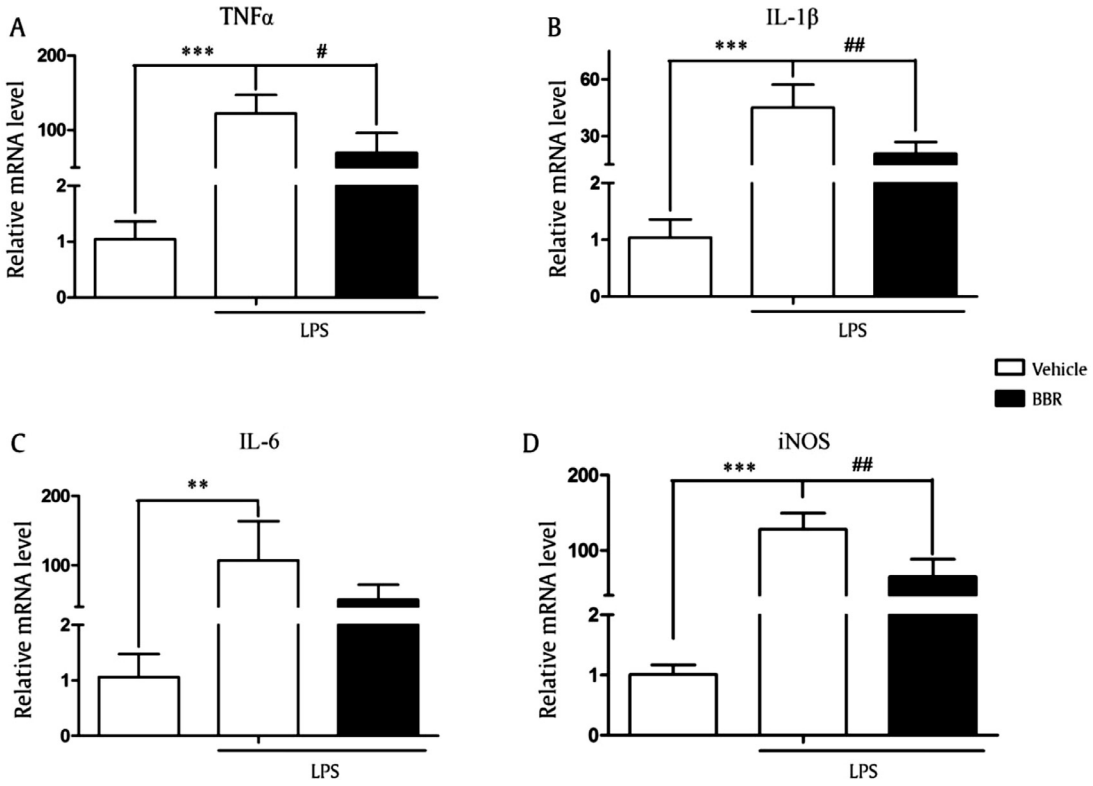

Fig. 2. Berberine repressed hepatic expression of inflammatory factor genes.
Expression levels of genes encoding inflammatory factors TNFα (A), IL-1β (B), IL-6 (C) and iNOS (D) in liver were determined. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* vehicle group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* LPS group.

Table 2
Levels of ALT, AST and AKP in serum.

Groups	ALT / (U·L^{-1})	AST / (U·L^{-1})	AKP / (U·L^{-1})
LPS	65.99 ± 7.41	54.42 ± 7.52	126.44 ± 3.9
Vehicle	57.39 ± 18.46	47.96 ± 5.01	101.63 ± 17.24*
LPS+BBR	73.31 ± 4.09	50.01 ± 2.52	101.46 ± 9.14**

$^{*}P < 0.05$; $^{**}P < 0.01$ *vs* LPS group.

3.4. BBR can reverse repression of CYP7A1 and CYP8B1 expression induced by LPS treatment

LPS treatment can induce the overexpression of TNFα and IL-1β and decrease the expression of CYP7A1 and CYP8B1 to 7.4% ($P = 0.021$, Fig. 4A) and 38.7% ($P < 0.001$, Fig. 4B), respectively, compared to the vehicle group, whereas it only slightly induced the expression of CYP27A1 and CYP7B1 (not significant, Fig. 4C and 4D). BBR treatment could significantly increase the mRNA expression levels of CYP7A1 and CYP8B1 to 220.9% ($P = 0.029$) and 208.2% ($P = 0.032$) compared with LPS group, respectively, but only slightly reduced the expression levels of CYP7B1 and CYP27A1 (not significant).

3.5. BBR modulates bile acid metabolism through NF-kB and MAPK signaling pathways

To explore whether the NF-kB and MAPK pathways mediate the BBR-mediated inhibition of the inflammatory response induced by LPS and the inhibition of cholesterol metabolism enzymes, the protein levels of p65 NF-κB and phosphorylated and total ERK, JNK, and p38 mitogen-activated protein kinases were determined by Western blotting. The protein level of CYP7A1 was reduced by LPS treatment and this phenotype was reversed by BBR treatment. LPS increased the expression of p65 NF-kB, indicating an inflammatory response, while BBR treatment decreased its expression. In the case of the MAPK signaling pathways, LPS increased the level of p-ERK protein, and BBR further elevated the levels of p-ERK and p-p38 MAPK, while neither LPS nor BBR treatment altered the expression of total ERK, JNK and p38 MAPK.

These results indicated that BBR may inhibit the LPS-induced inflammatory response and the inhibition of cholesterol metabolism via the NF-kB and MAPK pathways.

4. Discussion

BBR has been reported to have hepatic protection and NAFLD amelioration effects in different species, and several studies have demonstrated that the underlying mechanisms involve several pathways in the liver. An anti-inflammatory effect is one of the pathways accounting for the hepatic protective effect. BBR attenuates LPS-induced endometritis in mice by suppressing activation of the NF-κB signaling pathway (Fu et al., 2015), and BBR can also prevent an HIV protease inhibitor (amprenavir, atazanavir, lopinavir, and ritonavir)-induced inflammatory response by limiting ER stress (Zha et al., 2010). The activation of the AMPK

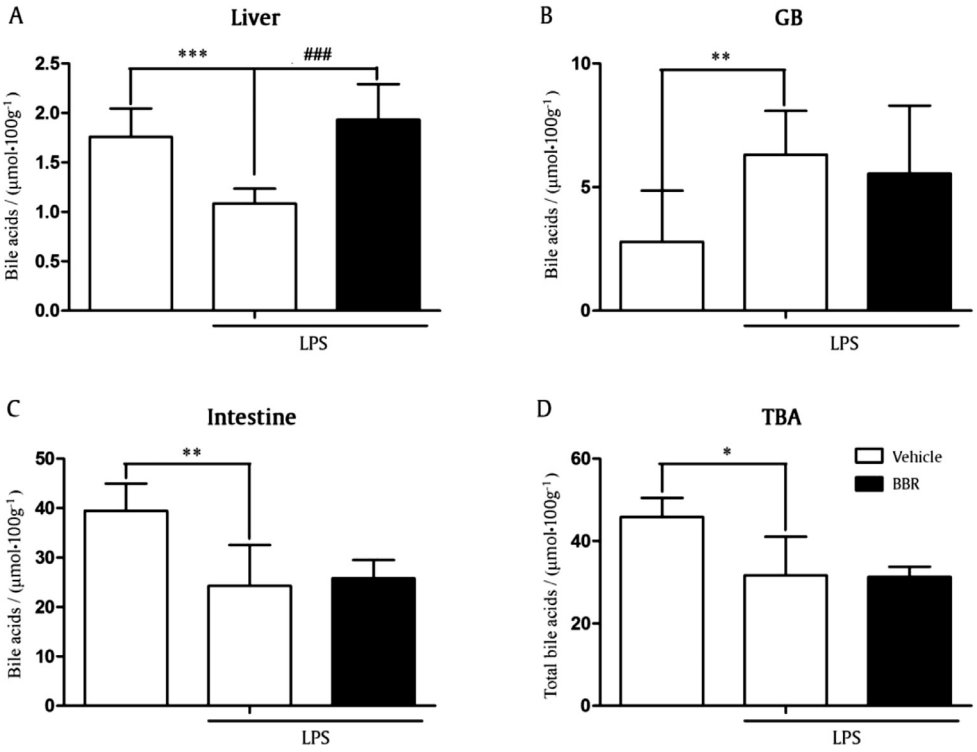

Fig. 3. Changes in bile acid pool induced by berberine treatment.
Liver, gallbladder and total intestine were collected at end of experiment and total bile acids were extracted and measured. Bile acids in liver (A), gallbladder (B), total intestine (C) and total bile acid pool (D) were calculated and expressed as μmol/100 g. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* vehicle group; $^{\#\#\#}P < 0.001$ *vs* LPS group.

pathway by BBR can also lead to suppressed pro-inflammatory responses in macrophages (Jeong et al., 2009). In a CCl_4-intoxicated mice model, BBR can inhibit production of TNFα, COX-2 and iNOS to offer hepatoprotective activity (Domitrovic, Jakovac, & Blagojevic, 2011). BBR can modulate the global hepatic mRNA and lncRNA expression profiles to ameliorate NAFLD, including lncRNA MRAK052686 and its associated gene Nrf2 (Yuan et al., 2015). Decreased methylation of the MTTP promoter can also alleviate fatty liver induced by a high-fat diet in rats (Chang et al., 2010). Here, we established an acute inflammatory model to evaluate the influence of BBR on LPS treated mice as well as to study cholesterol and bile acid homeostasis.

Bile acids are metabolized from cholesterol in the liver. CYP7A1 is the first and rate-limiting enzyme of the classical pathway that accounts for the majority of total bile acid synthesis, while CYP27A1 and CYP7B1 are the enzymes of the alternative pathway that accounts for the minority of total bile acid synthesis. Oxysterol can upregulate the expression of CYP7A1 through LXR to eliminate extra cholesterol, which is known as positive feedback regulation of bile acids. High FXR expression inhibits CYP7A1 expression in the pathway of negative feedback regulation of bile acid metabolism. Bile acids play an important role in lipid absorption and the modulation of cholesterol metabolism, and they are now considered a kind of metabolic regulation factor that can regulate lipid and glucose metabolism, as well as energy homeostasis. Moreover, bile acid metabolism is associated with various inflammatory diseases. However, there is still an argument whether bile acids can activate or inhibit NLRP3 inflammasome (Guo et al., 2016; Hao et al., 2017). Several studies have shown that BBR can modulate the metabolism of cholesterol and bile acid. In a hyperlipidemic rat model, Coptis alkaloids extract (CAE), which contains BBR, can upregulate the expression of CYP7A1 in a dose-dependent manner via activation of PPARα and down-regulation of FXR mRNA expression (Cao et al., 2012). In HepG2 cells, BBR can induce the expression of CYP7A1 and CYP27A1 in a dose-dependent manner. However, there are no studies on the relationship between the anti-inflammatory effect and upregulation of CYP7A1 expression induced by BBR (Gu et al., 2015).

Our results showed that LPS treatment can significantly inhibit the classical pathway of bile acid production and slightly induce the alternative pathway, shown by the inhibited expression of CYP7A1 and CYP8B1, and the slightly elevated expression of CYP27A1 and CYP7B1. Perhaps this is the body's reaction to the decrease in the bile acid pool. BBR treatment can achieve an obvious anti-inflammatory effect as revealed by the decreased levels of TNFα and IL-1β in the serum and reductions in their gene expression levels in the liver. These effects occur mainly via the activation of the MAPK signaling pathway and the inhibition of the NF-κB signaling pathway, which ultimately leads to the alleviation of the LPS-dependent inhibition of CYP7A1 and CYP8B1 expression to promote the production of bile acids in the liver. The alternative pathway and slightly induced CYP27A1 and CYP7B1 levels were also reversed by BBR treatment. These results revealed that an anti-inflammatory effect may be an important factor for modulating the enzymes responsible for cholesterol metabolism. In the long-term high fat-treated model, BBR may also induce the expression of CYP7A1 via inhibition of the inflammatory response.

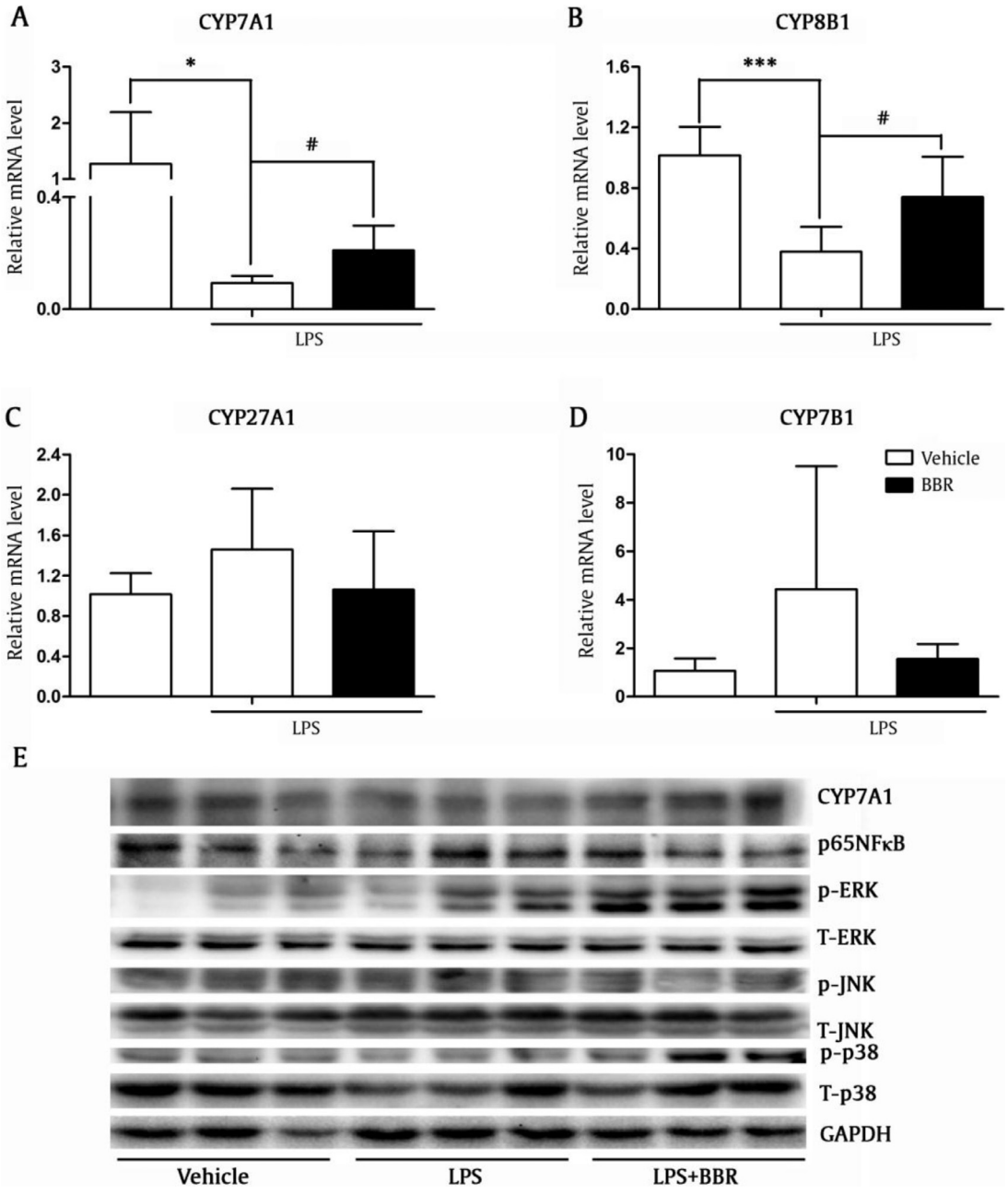

Fig. 4. Alteration of enzymes involved in cholesterol and bile acid metabolism, NF-κB and MAPK signaling pathways.
Classical and alternative pathways are main pathways of cholesterol and bile acid metabolism. Livers were collected at end of experiment, total RNA was extracted, and relative mRNA levels of CYP7A1 (A), CYP8B1 (B), CYP27A1 (C) and CYP7B1 (D) were determined by qRT-PCR. NF-κB and MAPK signaling pathways (E) were measured by Western blotting. $^{*}P < 0.05$, $^{***}P < 0.001$ *vs* vehicle group; $^{\#}P < 0.05$ *vs* LPS group.

5. Conclusion

In conclusion, BBR showed a striking anti-inflammatory effect, alleviated the LPS-dependent decrease in CYP7A1 and CYP8B1 expression, and promoted the production of bile acids in liver, mainly via activation of the MAPK signaling pathway and inhibition of the NF-κB signaling pathway. The effect of a high fat diet and BBR treatment on CYP7A1 expression and the metabolism of cholesterol and bile acid should be further evaluated.

Conflict of interest

The authors declare no competing financial interests.

Acknowledgements

This project was supported by grants from the National Natural Science Foundation of China (81503139, 81573495) and the Key Technology Projects of China “Creation of New Drugs” (2017ZX09301013).

References

Alkhouri, N., Carter-Kent, C., & Feldstein, A. E. (2011). Apoptosis in nonalcoholic fatty liver disease: Diagnostic and therapeutic implications. *Expert Review of Gastroenterology & Hepatology, 5*(2), 201.

Basu, S., Rosenzweig, K. R., Youmell, M., & Price, B. D. (1998). The DNA-dependent protein kinase participates in the activation of NF-κB following DNA damage. *Biochemical and Biophysical Research Communications, 247*(1), 79–83.

Cao, Y., Bei, W., Hu, Y., Cao, L., Huang, L., Wang, L., Luo, D., Chen, Y., Yao, X., He, W., Liu, X., & Guo, J. (2012). Hypocholesterolemia of *Rhizoma Coptidis* alkaloids is related to the bile acid by up-regulated CYP7A1 in hyperlipidemic rats. *Phytomedicine, 19*(8–9), 686–692.

Chandel, N. S., Trzyna, W. C., McClintock, D. S., & Schumacker, P. T. (2000). Role of oxidants in NF-κB activation and TNF-α gene transcription induced by hypoxia and endotoxin. *The Journal of Immunology, 165*(2), 1013–1021.

Chang, X., Yan, H., Fei, J., Jiang, M., Zhu, H., Lu, D., & Gao, X. (2010). Berberine reduces methylation of the MTTP promoter and alleviates fatty liver induced by a high-fat diet in rats. *Journal of Lipid Research, 51*(9), 2504–2515.

Chu, M., Ding, R., Chu, Z. Y., Zhang, M. B., Liu, X. Y., Xie, S. H., Zhai, Y. J., & Wang, Y. D. (2014). Role of berberine in anti-bacterial as a high-affinity LPS antagonist binding to TLR4/MD-2 receptor. *BMC Complementary and Alternative Medicine, 14*(1), 89.

Dikopoulos, N., Weidenbach, H., Adler, G., & Schmid, R. (2003). Lipopolysaccharide represses cholesterol 7-alpha hydroxylase and induces binding activity to the bile acid response element II. *European Journal of Clinical Investigation, 33*(1), 58–64.

Domitrovic, R., Jakovac, H., & Blagojevic, G. (2011). Hepatoprotective activity of berberine is mediated by inhibition of TNF-alpha, COX-2, and iNOS expression in CCl(4)-intoxicated mice. *Toxicology, 280*(1-2), 33–43.

Duez, H., Van Der Veen, J. N., Duhem, C., Pourcet, B., Touvier, T., Fontaine, C., Derudas, B., Baugé, E., Havinga, R., & Bloks, V. W. (2008). Regulation of bile acid synthesis by the nuclear receptor Rev-erbα. *Gastroenterology, 135*(2), 689–698 e685.

Feingold, K. R., Spady, D. K., Pollock, A. S., Moser, A. H., & Grunfeld, C. (1996). Endotoxin, TNF, and IL-1 decrease cholesterol 7 alpha-hydroxylase mRNA levels and activity. *Journal of Lipid Research, 37*(2), 223–228.

Fitzgerald, D., Meade, K., McEvoy, A., Lillis, L., Murphy, E., MacHugh, D., & Baird, A. (2007). Tumour necrosis factor-α (TNF-α) increases nuclear factor κB (NFκB) activity in and interleukin-8 (IL-8) release from bovine mammary epithelial cells. *Veterinary Immunology and Immunopathology, 116*(1), 59–68.

Fu, K., Lv, X., Li, W., Wang, Y., Li, H., Tian, W., & Cao, R. (2015). Berberine hydrochloride attenuates lipopolysaccharide-induced endometritis in mice by suppressing activation of NF-κB signal pathway. *International Immunopharmacology, 24*(1), 128–132.

Fu, Z. D., Csanaky, I. L., & Klaassen, C. D. (2012). Gender-divergent profile of bile acid homeostasis during aging of mice. *PLoS One, 7*(3), e32551.

Gao, M. Y., Chen, L., Yang, L., Yu, X., Kou, J. P., & Yu, B. Y. (2014). Berberine inhibits LPS-induced TF procoagulant activity and expression through NF-kappaB/p65, Akt and MAPK pathway in THP-1 cells. *Pharmacological Reports, 66*(3), 480–484.

Goodwin, B., Watson, M. A., Kim, H., Miao, J., Kemper, J. K., & Kliewer, S. A. (2003). Differential regulation of rat and human CYP7A1 by the nuclear oxysterol receptor liver X receptor-α. *Molecular Endocrinology, 17*(3), 386–394.

Gu, S., Cao, B., Sun, R., Tang, Y., Paletta, J. L., Wu, X. L., Liu, L., Zha, W., Zhao, C., & Li, Y. (2015). A metabolomic and pharmacokinetic study on the mechanism underlying the lipid-lowering effect of orally administered berberine. *Molecular BioSystems, 11*(2), 463–474.

Guo, C., Xie, S., Chi, Z., Zhang, J., Liu, Y., Li, Z., Zheng, M., Xue, Z., Xia, D., & Ke, Y. (2016). Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome. *Immunity, 45*(4), 802–816.

Gupta, S., Pandak, W. M., & Hylemon, P. B. (2002). LXRα is the dominant regulator of CYP7A1 transcription. *Biochemical and Biophysical Research Communications, 293*(1), 338–343.

Hao, H., Cao, L., Jiang, C., Che, Y., Zhang, S., Takahashi, S., Wang, G., & Gonzalez, F. J. (2017). Farnesoid X receptor regulation of the NLRP3 inflammasome underlies cholestasis-associated sepsis. *Cell Metabolism, 25*(4), 856–867.

Henkel, A. S., Anderson, K. A., Dewey, A. M., Kavesh, M. H., & Green, R. M. (2011). A chronic high-cholesterol diet paradoxically suppresses hepatic CYP7A1 expression in FVB/NJ mice. *Journal of Lipid Research, 52*(2), 289–298.

James, O. F., & Day, C. P. (1998). Non-alcoholic steatohepatitis (NASH): A disease of emerging identity and importance. *Journal of Hepatology, 29*(3), 495–501.

Jeong, H. W., Hsu, K. C., Lee, J. W., Ham, M., Huh, J. Y., Shin, H. J., Kim, W. S., & Kim, J. B. (2009). Berberine suppresses proinflammatory responses through AMPK activation in macrophages. *American Journal of Physiology-Endocrinology and Metabolism, 296*(4), E955–E964.

Kong, B., Wang, L., Chiang, J. Y., Zhang, Y., Klaassen, C. D., & Guo, G. L. (2012). Mechanism of tissue-specific farnesoid X receptor in suppressing the expression of genes in bile-acid synthesis in mice. *Hepatology, 56*(3), 1034–1043.

Lou, T., Zhang, Z., Xi, Z., Liu, K., Li, L., Liu, B., & Huang, F. (2011). Berberine inhibits inflammatory response and ameliorates insulin resistance in hepatocytes. *Inflammation, 34*(6), 659–667.

Ma, K., Xiao, R., Tseng, H. T., Shan, L., Fu, L., & Moore, D. D. (2009). Circadian dysregulation disrupts bile acid homeostasis. *PLoS One, 4*(8) e6843–e6843.

Mo, C., Wang, L., Zhang, J., Numazawa, S., Tang, H., Tang, X., Han, X., Li, J., Yang, M., & Wang, Z. (2014). The crosstalk between Nrf2 and AMPK signal pathways is important for the anti-inflammatory effect of berberine in LPS-stimulated macrophages and endotoxin-shocked mice. *Antioxidants & Redox Signaling, 20*(4), 574–588.

Polyzos, S., Kountouras, J., & Zavos, C. (2009). The multi-hit process and the antagonistic roles of tumor necrosis factor-alpha and adiponectin in non alcoholic fatty liver disease. *Hippokratia, 13*(2), 127.

Qin, H., Wilson, C. A., Lee, S. J., Zhao, X., & Benveniste, E. N. (2005). LPS induces CD40 gene expression through the activation of NF-κB and STAT-1α in macrophages and microglia. *Blood, 106*(9), 3114–3122.

Renard, P., Zachary, M. D., Bougelet, C., Mirault, M. E., Haegeman, G., Remacle, J., & Raes, M. (1997). Effects of antioxidant enzyme modulations on interleukin-1-induced nuclear factor kappa B activation. *Biochemical Pharmacology, 53*(2), 149–160.

Russell, D. W. (2009). Fifty years of advances in bile acid synthesis and metabolism. *Journal of Lipid Research, 50*(Supplement), S120–S125.

Saha, R. N., Jana, M., & Pahan, K. (2007). MAPK p38 regulates transcriptional activity of NF-κB in primary human astrocytes via acetylation of p65. *The Journal of Immunology, 179*(10), 7101–7109.

Sinal, C. J., Tohkin, M., Miyata, M., Ward, J. M., Lambert, G., & Gonzalez, F. J. (2000). Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell, 102*(6), 731–744.

Tang, J., Luo, K., Li, Y., Chen, Q., Tang, D., Wang, D., & Xiao, J. (2015). Capsaicin attenuates LPS-induced inflammatory cytokine production by upregulation of LXRalpha. *International Immunopharmacology, 28*(1), 264–269.

Thomas, C., Pellicciari, R., Pruzanski, M., Auwerx, J., & Schoonjans, K. (2008). Targeting bile-acid signalling for metabolic diseases. *Nature Reviews Drug Discovery, 7*(8), 678–693.

Xie, W., Gu, D., Li, J., Cui, K., & Zhang, Y. (2011). Effects and action mechanisms of berberine and *Rhizoma Coptidis* on gut microbes and obesity in high-fat diet-fed C57BL/6J mice. *PloS One, 6*(9), e24520.

Xu, Z., Tavares-Sanchez, O. L., Li, Q., Fernando, J., Rodriguez, C. M., Studer, E. J., Pandak, W. M., Hylemon, P. B., & Gil, G. (2007). Activation of bile acid biosynthesis by the p38 mitogen-activated protein kinase (MAPK) hepatocyte nuclear factor-4α phosphorylation by the p38 MAPK is required for cholesterol 7α-hydroxylase expression. *Journal of Biological Chemistry, 282*(34), 24607–24614.

Yuan, X., Wang, J., Tang, X., Li, Y., Xia, P., & Gao, X. (2015). Berberine ameliorates nonalcoholic fatty liver disease by a global modulation of hepatic mRNA and lncRNA expression profiles. *Journal of Translational Medicine, 13*(1), 1–11.

Zha, W., Liang, G., Xiao, J., Studer, E. J., Hylemon, P. B., Pandak, W. M., Jr, Wang, G., Li, X., & Zhou, H. (2010). Berberine inhibits HIV protease inhibitor-induced inflammatory response by modulating ER stress signaling pathways in murine macrophages. *PLoS One, 5*(2), e9069.

Zhang, X., Zhao, Y., Zhang, M., Pang, X., Xu, J., Kang, C., Li, M., Zhang, C., Zhang, Z., & Zhang, Y. (2012). Structural changes of gut microbiota during berberine-mediated prevention of obesity and insulin resistance in high-fat diet-fed rats. *PLoS One, 7*(8), e42529.