AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Role of Inflammatory and Oxidative Stress, Cytochrome P450 2E1, and Bile Acid Disturbance in Rat Liver Injury Induced by Isoniazid and Lipopolysaccharide Cotreatment

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Isoniazid (INH) remains the core drug in tuberculosis management, but serious hepatotoxicity and potentially fatal liver injury continue to accompany INH consumption. Among numerous theories that have been established to explain INH-induced liver injury, an inflammatory stress theory has recently been widely used to explain the idiosyncrasy. Inflammatory stress usually sensitizes tissues to a drug's toxic consequences. Therefore, the present study was conducted to verify whether bacterial lipopolysaccharide (LPS)-induced inflammation may have a role in enhancing INH hepatotoxicity. While single INH or LPS administration showed no major toxicity signs, INH-LPS cotreatment intensified liver toxicity. Both blood biomarkers and histological evaluations clearly showed positive signs of severe liver damage accompanied by massive necrosis, inflammatory infiltration, and hepatic steatosis. Furthermore, elevated serum levels of bile acid associated with the repression of bile acid synthesis and transport regulatory parameters were observed. Moreover, the principal impact of cytochrome P450 2E1 (CYP2E1) on INH toxicity could be anticipated, as its protein expression showed enormous increases in INH-LPS-cotreated animals. Furthermore, the crucial role of CYP2E1 in the production of reactive oxygen species (ROS) was clearly obvious in the repression of hepatic antioxidant parameters. In summary, these results confirmed that this LPS-induced inflammation model might prove valuable in revealing the hepatotoxic mechanisms of INH and the crucial role played by CYP2E1 in the initiation and propagation of INH-induced liver damage, information which could be very useful to clinicians in understanding the pathogenesis of drug-induced liver injury.

Isoniazid (INH), since its introduction in the year 1952, still serves as a frontline drug in tuberculosis treatment (1). Despite the fact that INH has been widely used as a first-line antitubercular agent (2, 3), its therapeutic value is usually accompanied by severe hepatotoxicity and lethal hepatic injury (4, 5). Although the pathophysiology of INH-induced liver injury might vary, the toxicity features of the drug, including hepatocellular steatosis, necrosis, and inflammatory infiltration, are nearly consistent (6, 7).

Even though extensive studies expounding INH toxicity have been carried out, the exact mechanism of INH hepatotoxicity remains controversial. Among numerous established theories, an inflammatory stress theory has recently been widely used to explain the idiosyncrasy. One hypothesis is that inflammatory stress increases sensitivity to drug-induced liver injury (DILI) (8, 9). In this theory, an incidence of systemic inflammation might reduce the xenobiotic toxicity threshold, which could be easily accomplished by coadministration with an inflammatory agent (10), thus promoting drug toxicity. Lipopolysaccharide (LPS) is an outer cell wall membrane constituent of Gram-negative bacteria that has been comprehensively studied as an inflammatory agent with a major role in bacterial infections (11, 12). Previous studies have suggested that LPS might intensify DILI (13–15), and, hence, a possible cornerstone role for LPS in INH-induced hepatotoxicity might be assumed.

Oxidative stress, generated from the accumulation of reactive oxygen species (ROS), may be potentiated by different factors, including drugs and inflammation (16, 17). In addition, oxidative stress plays a major role in several types of hepatic injury (18). Furthermore, with cytochrome P450 2E1 (CYP2E1) playing a major role in drug metabolism and the pathophysiology of DILI (19) and being considered a principal element in human susceptibility to chemical toxins (20), it plays a central part in oxidative stress, production of ROS, and hepatotoxic injury. Moreover, a previous report proposed that hepatic CYP2E1 plays a fundamental role in the propagation of INH-induced hepatotoxicity, mainly throughout ROS generation (21). Nevertheless, a full understanding of CYP2E1 as a major hepatotoxin-forming, catalyzing enzyme and its influence on INH-induced liver damage has not been completely achieved.

Studies of the hepatotoxic mechanisms of INH were previously conducted using different animal models; however, results were accompanied by the absence of certain features of INH toxicity, i.e., delayed onset or inconsistency in severity compared to that in hu-

Received 20 April 2016 **Returned for modification** 25 May 2016
Accepted 11 June 2016

Accepted manuscript posted online 20 June 2016

Citation Hassan HM, Guo H, Yousef BA, Guerram M, Hamdi AM, Zhang L, Jiang Z. 2016. Role of inflammatory and oxidative stress, cytochrome P450 2E1, and bile acid disturbance in rat liver injury induced by isoniazid and lipopolysaccharide cotreatment. Antimicrob Agents Chemother 60:5285–5293. doi:10.1128/AAC.00854-16.

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mans (3). Despite the lack of success in reproducing the same clinical features of INH hepatotoxicity, our group in a previous study successfully established an animal model that could fill the gap associated with understanding the whole picture. In that study, Su et al. (22) concluded that LPS inflammatory activity positively sensitized hepatic cells toward INH-induced liver injury.

Therefore, due to the potential enhancement activity of LPS-induced inflammation in drug toxicities, the present study aimed to explore and verify whether LPS might enhance INH hepatotoxicity and to investigate the underlying mechanisms that are possibly responsible for this enhancement; this was done by establishing a suitable animal model that could predict some idiosyncratic reactions, which could then be used to identify potential problems earlier and to direct appropriate preventative actions.

MATERIALS AND METHODS

Drugs, chemicals, and antibodies. INH (CAS number 54-85-3; analytical standard, ≥99%) and LPS (lot number 025M4128V; derived from *Escherichia coli* serotype O128:B12, source strain CDC2440-69) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other reagents were commercially available and of high analytical grade.

Animals. Male Sprague-Dawley (SD) rats (180 to 200 g) were obtained from Shanghai Lingchang Biological Technology Co., Ltd. (Shanghai, China). All experimental procedures were conducted in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals. Rats were housed in controlled environmental conditions (23 ± 1°C, 55% ± 5% relative humidity, 12-h light–12-h dark cycle) with free access to food and water *ad libitum*. Animals were acclimatized for 1 week before the experiments were conducted.

Treatment protocol. Thirty-six rats were divided into six groups of six animals each. Group I served as the control; group II received LPS (2.0 mg/kg intravenously), whereas both group III and group IV received INH (200 and 400 mg/kg, respectively, intragastrically), while group V and group VI received INH at the above-mentioned dosages, respectively, plus LPS (2.0 mg/kg).

INH was intragastrically administered for 14 consecutive days, while LPS was given as an intravenous bolus dose at day 14, 2 h before the INH dose. Drug doses were selected based on previous studies (22–24), with slight modification. After the last INH dose, the rats were sacrificed; blood samples and liver sections were collected for further biological evaluation. All experimental procedures were ethically approved by both China Pharmaceutical University and the ethical committees of the National Drug Screening Centre, Nanjing, China.

Serum and liver biological parameters. Serum alanine transaminase (ALT), aspartate transaminase (AST), total bile acids (TBA), total bilirubin (TBil), gamma-glutamyl transferase (γGGT), triglyceride (TG), and total cholesterol (TC) levels were measured with an HITAC7170A automatic analyzer (Hitachi, Japan) in accordance with standard spectrophotometric methods. Hepatic TG and TC were analyzed using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Histology evaluation. Liver slices were fixed in 10% paraformaldehyde solution and embedded in paraffin wax. Sections were cut at 5-mm thickness and stained with hematoxylin-eosin. Slides were coded, randomized, and evaluated by a pathophysiologist using light microscopy.

Measurement of antioxidant enzymes and MDA levels. Superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA), and total antioxidant capacity (T-AOC) assays were carried out using appropriate kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), in accordance with the manufacturer's instructions.

Real-time quantitative PCR (RT-PCR). Total RNA was extracted with TRIzol (Vazyme Biotech, Nanjing, China), and cDNA synthesis was performed using the PrimeScript RT master mix (TaKaRa Biotechnology, Dalian, China) according to the manufacturer's instructions. Quantitative reverse transcription-PCR was performed using the SYBR green PCR master mix (Vazyme Biotech, Nanjing, China). Gene expression was evaluated using the $\Delta\Delta C_T$ method, with the GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene serving as a reference.

Western blotting. Radioimmunoprecipitation assay (RIPA) buffer and phosphatase and protease inhibitors (Vazyme Biotech, Nanjing, China) were used for liver protein extraction. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)-separated proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA). After nonspecific blocking with 5% skim milk for an hour, the membranes were probed with primary antibody (1:500 to 1:1,000) in 5 ml blocking buffer overnight at 4°C. The membranes were then washed four times with Tris-buffered saline with Tween 20 (TBST) buffer and incubated with suitable horseradish peroxidase (HRP)-conjugated secondary antibody. Membranes were further washed four times with TBST, incubated with an ECL solution (Millipore, USA), and digitally imaged with a charge-coupled device (CCD) camera.

TUNEL staining. For the detection of apoptosis, paraffin-embedded sections were stained by a terminal dUTP nick-end labeling (TUNEL) technique using a TUNEL detection kit (KeyGEN BioTECH, Nanjing, China) according to the manufacturer's protocols.

Statistics. Results are expressed as means ± standard deviations; one-way analysis of variance (ANOVA) and the Student-Newman-Keuls *post hoc* test were used to determine differences between the results for treated and control animals. The criterion for significance was a P value of <0.05 for all comparisons.

RESULTS

LPS had synergistic effects on INH-induced hepatic injury. Body weight was significantly reduced by the INH-LPS combination (Fig. 1A). In the meantime, serum AST, TBA, TBil, and γGGT levels were significantly raised compared to those of INH-only groups, with the highest increase observed with the INH (400 mg/kg)-LPS cotreatment (Fig. 1B and C). Interestingly, the serum ALT level, as a major marker of liver injury, was significantly reduced in INH-treated animals, while in the INH-LPS-cotreated animals, it initially increased but was still below the control group level (Fig. 1B). In addition, both serum and hepatic TG and TC levels showed no changes compared to those of the control group after administration of INH or LPS alone, whereas the combined drugs significantly elevated TG and TC levels in a dose-dependent manner (Fig. 1D). Moreover, histological assessment revealed that administration of either LPS or INH alone had a minor effect on normal liver histology (Fig. 2A), while intense micro- and macrovesicular steatosis, severe hepatocellular necrosis, and inflammatory infiltration were observed in INH-LPS-cotreated rats, results which are associated with a significant difference in their histological scores (Fig. 2B).

Effects on oxidative stress. Both INH and LPS are known individually to generate, induce, and potentiate oxidative stress (25–27). In order to evaluate whether LPS could potentiate INH-induced oxidative stress, we analyzed certain hepatic parameters involved in ROS production. As shown in Fig. 3, we found that the INH-LPS combination marginally decreased the hepatic SOD level, with a significant reduction observed in INH (400 mg/kg)-LPS-cotreated rats, while a minor, insignificant reduction was observed in the overall liver T-AOC. Meanwhile, analysis of MDA, as one of the major lipid peroxidation parameters, showed reduced levels in the groups treated with INH alone but an increase in INH-LPS-cotreated groups, although this elevation was insignificant. For hepatic GSH, the major intracellular antioxidant defense mechanism, there was an increase in the group treated with LPS

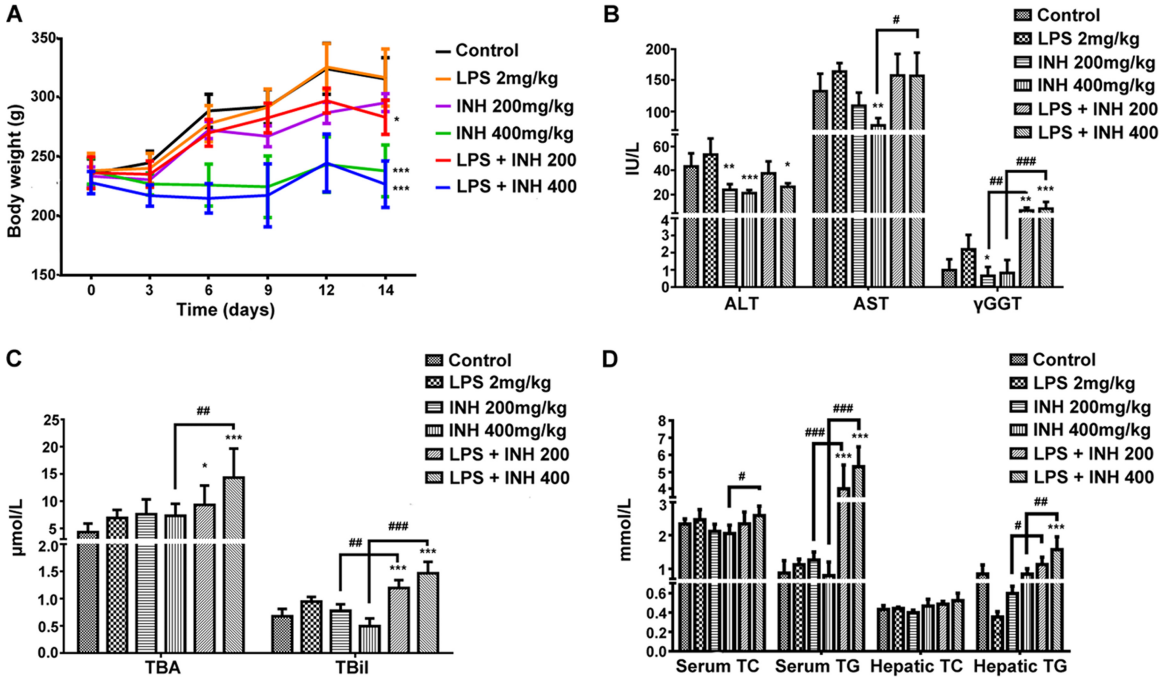

FIG 1 Liver injury parameters induced by INH-LPS cotreatment. Rats were treated with 200 or 400 mg/kg INH for 14 consecutive days, and at day 14, they received 2 mg/kg LPS, followed by INH 2 h later. (A) Effects on body weight; (B) changes in serum ALT, AST, and γGGT levels; (C) influence on serum TBA and TBil levels; (D) variations in both serum and hepatic lipid profiles. Data are given as means ± SD, $n = 6$ for each bar. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (versus results for the control). #, $P < 0.05$; ##, $P < 0.01$; ###, $P < 0.001$ (versus results in the absence of LPS).

alone, in contrast to a significant gradual reduction among the other groups, with the greatest inhibition occurring in INH-LPS-cotreated animals. These results highlight the positive role that is played by oxidative stress in INH hepatotoxicity, with LPS augmenting this action.

Gene profile associated with INH-induced hepatotoxicity. As the total bile acid levels in serum were significantly elevated following administration of INH-LPS cotreatment, we analyzed the expression of certain genes that participate in the synthesis and regulation of bile acids. Our results demonstrated that expression

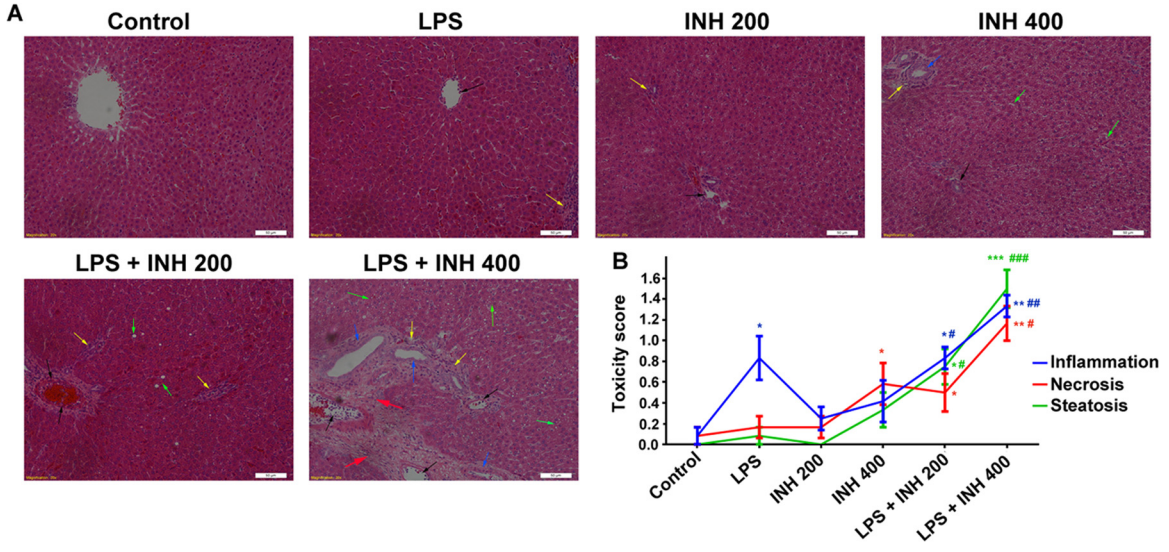

FIG 2 Liver histopathological examination after INH-LPS cotreatment. (A) Liver slices were collected and subjected to staining with hematoxylin and eosin. The control group shows normal hepatocyte architecture, the animals treated with either LPS or INH alone show minor alterations, and INH-LPS-cotreated animals show severe toxicity symptoms. Inflammatory cells (black arrows), inflammatory infiltration (yellow arrows), bile duct hyperplasia (blue arrows), micro- and macrovesicular steatosis (green arrows), and massive necrosis and hepatocellular structure loss (red arrows) are indicated. (B) INH hepatotoxicity score in the absence or presence of LPS. Data are given as means ± SD, $n = 6$ for each bar. *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$ (versus results for the control); #, $P < 0.05$; ##, $P < 0.01$; ###, $P < 0.001$ (versus results in the absence of LPS).

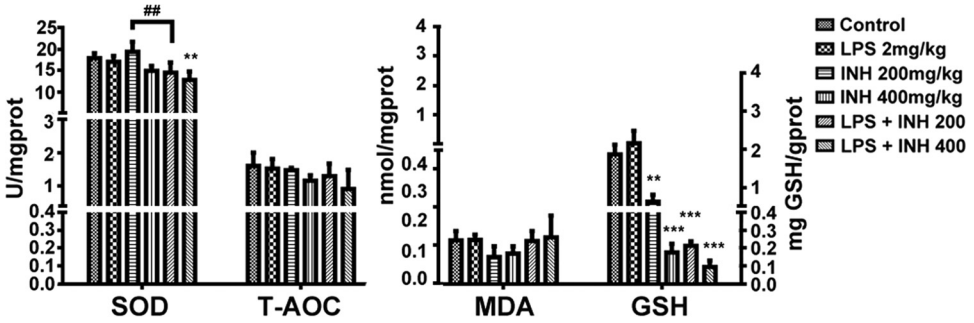

FIG 3 INH and LPS cause modifications in oxidative stress and hepatic antioxidant defense mechanisms. Changes in SOD, T-AOC, MDA, and GSH levels were measured by their respective kits. Data are given as means ± SD, $n = 6$ for each bar. **, $P < 0.01$; ***, $P < 0.001$ (versus results for the control); ##, $P < 0.01$ (versus results in the absence of LPS).

levels of both farnesoid X receptor (FXR) and small heterodimer partner (SHP) genes were gradually reduced in groups treated with INH alone, while LPS coadministration severely diminished the FXR and SHP gene expression levels (Fig. 4A). This diminished FXR activity triggered bile acid overproduction due to the loss of FXR control of the bile acid synthesis pathway. Interestingly, expression levels of genes responsible for bile acid synthesis, namely, the CYP7A1, CYP27A1, and CYP8B1 genes, were significantly decreased in INH-LPS-cotreated animals, although CYP7A1 gene expression showed a significant dose-dependent elevation in the group treated with INH alone, which was consistent with FXR reduction (Fig. 4B). The reduction in gene expression levels appearing in INH-LPS-cotreated animals might occur as a negative-feedback consequence following the initial increase in bile acid level in hepatocytes (28). In the meantime, the reduction in both CYP7A1 and CYP8B1 correlated with increases in hepatic TG and TC, as these enzymes are responsible for bile acid synthesis from cholesterol (29) and thus reduction of either one or both of these enzymes will definitely elevate the TG and TC levels.

On the other hand, expression levels of bile acid transporters, namely, bile salt export pump (BSEP), Na^+-taurocholate cotransporting polypeptide (NTCP), and multidrug resistance-associated protein 2 (MRP_2), were also significantly reduced in INH-LPS-cotreated groups compared to those of the control and INH-only animals (Fig. 4C). The reduction of these bile acid transporters, mainly as a result of combined activity with LPS, allowed accumulation of toxic bile acids in hepatocytes, which triggered cellular necrosis and the release of liver cellular contents, including bile acid, into serum, thus explaining the increased serum TBA level.

Even though the participation of inflammatory mediators and the immune system in the pathogenesis of INH-induced liver toxicity was inconsistent and varied, our results showed the positive role played by inflammation and the immune system in the propagation of INH toxicity. Figure 4D illustrates that both tumor necrosis factors alpha (TNF-α) and interleukin 6 (IL-6) showed incremental increases in expression in INH-LPS-cotreated rats, even higher than in the LPS-only group.

Researchers are familiar with the cornerstone role played by CYP2E1 in INH toxicity. Unexpectedly, CYP2E1 gene expression was severely diminished in almost all of the animal groups compared to the control (Fig. 4E), with more pronounced decreases in INH-LPS-cotreated animals. On the other hand, peroxisome proliferator-activated receptor alpha (PPARα), which is highly expressed in hepatocytes and responsible for lipid metabolism, showed a progressive decrease among the groups of treated animals, with the highest reduction observed after administration of the INH-LPS combination (Fig. 4F).

Western blot assessment of relevant protein expression. In correlation with their gene expression levels, reductions in the expression of FXR, CYP8B1, BSEP, and NTCP proteins are shown in Fig. 4G. In contrast, CYP2E1 protein expression was progressively elevated among the treated groups, with the greatest increase in the INH-LPS-cotreated groups. This finding further supports the role of CYP2E1 in explaining INH hepatotoxicity. In addition, significant downregulation of PPARα protein expression was also noticed, especially with a 400-mg INH dose combined with LPS.

Apoptosis in INH hepatotoxicity. Apoptotic induction is still considered a major pathway for INH toxicity (30). Therefore, we tested the potential of INH-induced apoptosis. We found that hepatic cleavage caspase-3 protein levels, as an indicator of cellular apoptosis, were provoked by the INH-LPS combination, while INH-only-treated groups showed minor protein levels (Fig. 5A). For further apoptotic confirmation, a TUNEL assay was conducted using liver slices. Results indicated that neither LPS-only treatment nor INH-only treatment led to apoptosis, but when they were combined, a positive TUNEL test indicating apoptosis was observed (Fig. 5B).

DISCUSSION

Progress in finding an exact mechanism for and establishing a suitable animal model of INH-induced hepatotoxicity has faced many obstacles (31). In accordance with this challenge, the present study aimed to prove our hypothesis that coexposure to INH and LPS intensifies INH-induced liver injury.

Previous studies have reported that exposure to low, injury-free LPS concentrations that are unable to cause tissue or cellular damage increases drug intoxication liabilities (32, 33). Therefore, animal models that follow the inflammatory stress theory, in which nontoxic LPS doses potentiate drug toxicities, were of great help in mechanistically elaborating a general picture of DILI.

This study was conducted entirely with male rats and followed our 14-day protocol; liver injury was confirmed by blood biochemical analysis. Increases in transaminase levels have long been considered the major signs of INH-induced hepatotoxicity, with up to 20% of patients usually suffering from elevated ALT levels (34); in contrast with those previous reports, we observed that

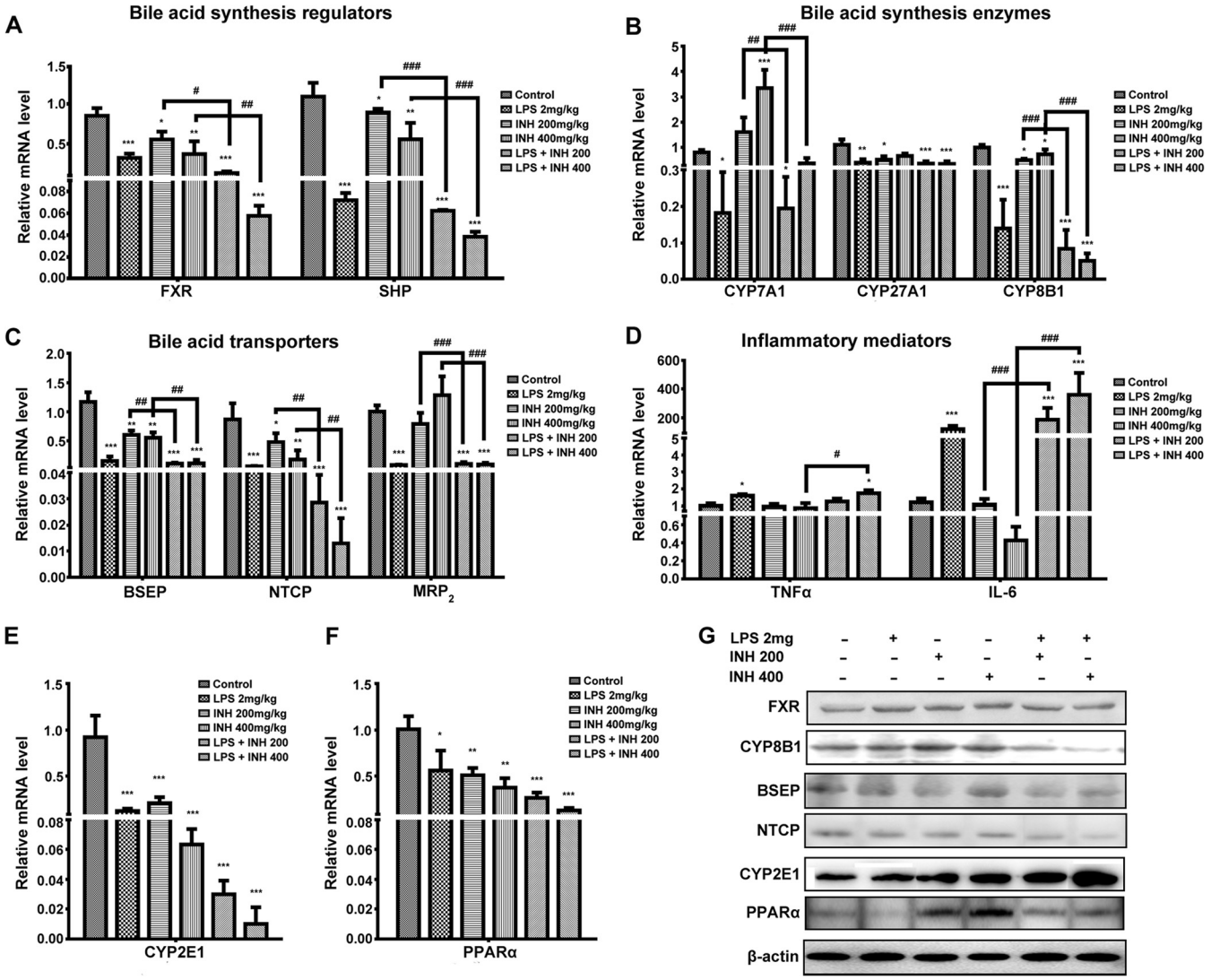

FIG 4 Effects of INH administration on different targeted genes and proteins in the presence or absence of LPS. (A) Expression of bile acid regulators FXR and SHP. (B) Expression of bile acid synthesis enzymes CYP7A1, CYP27A1, and CYP8B1. (C) Expression of bile acid transporters BSEP, NTCP, and MRP_2. (D) Expression of inflammatory mediators TNF-α and IL-6. (E) Expression of CYP2E1. (F) Expression of PPARα. Data are given as means ± SD, $n = 6$ for each bar. *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$ (versus results for the control); #, $P < 0.05$; ##, $P < 0.01$; ###, $P < 0.001$ (versus results in the absence of LPS). The GAPDH gene was set as a reference. (G) Immunoblotting analysis of different targeted proteins following INH-LPS cotreatment, with β-actin considered a loading control.

either acute (1-week) or subchronic (2-week) administration of INH led to reductions in ALT, AST, and alkaline phosphatase (ALP). On the other hand, increased time exposure to INH (4 weeks) was characterized by significant and severe increases in transaminase levels (data not shown). Some previously mentioned results (23, 35, 36) were in line with our findings, in which serum and hepatic levels of ALT and AST were reduced. This might be correlated with the duration of INH administration; increased exposure time to INH will elevate transaminase levels, although both decreased and increased levels are considered biomarkers for INH-induced liver injury. Nevertheless, a diagnosis of INH hepatotoxicity that depends solely on the serum level of transaminases may be inaccurate in accordance with these findings. A finding of INH hepatotoxicity was further fortified by histopathological evaluation of liver samples, which showed that massive necrosis and steatosis associated with inflammatory cell infiltrations occurred in INH-LPS-cotreated animal groups. The massive necrosis phenomenon that was seen might be attributed to INH-induced lipid peroxidation (7), which was enhanced by LPS inflammatory action. In line with this, Sarich et al. previously reported that INH caused hepatic necrosis and steatosis in rabbits (37).

Blocking the FXR regulatory role of the bile acid synthesis cascade from cholesterol resulted in the elevation of the bile acid level (29, 38). Bile acid transporters, namely, BSEP, NTCP, and MRP_2, play a key role in bile acid homeostasis (39, 40), while inhibition of the bile acid transporters BSEP and NTCP was suggested to participate in INH-induced liver injury pathogenesis (41). Our preceding study (22) showed that the INH-LPS combination elevated the expression of the bile acid-synthesizing enzyme (CYP7A1) while the expression of transporters (BSEP, NTCP, and MRP_2) was significantly reduced. Due to treatment duration differences,

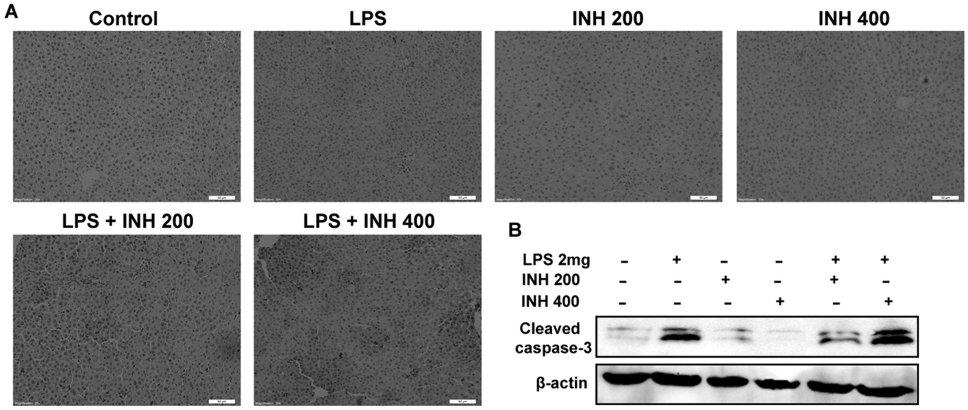

FIG 5 INH-LPS cotreatment causes apoptosis. (A) A TUNEL assay was conducted on liver slices, and treatment with either LPS or INH alone showed no apoptotic effects, while INH-LPS cotreatment caused obvious positive TUNEL results (intensity of color indicating apoptotic action). (B) Representative Western blot analysis of cleaved caspase-3, with β-actin considered a loading control.

we could determine the exact effect of INH-LPS in relation to bile acids as follows: in response to LPS-induced inflammation and released inflammatory cytokines, expression of the key regulatory transcriptional factor, the FXR gene, was repressed, causing increased production of bile acids. In the meantime, inflammation induced the rapid reduction in bile acid transporters, which caused the elevation and entrapment of bile acids in hepatocytes (42–45). This massive accumulation of bile acids in hepatocytes might initiate a negative-feedback mechanism that results in the observed gene expression reduction. Simultaneously, accumulation of toxic intracellular bile acid levels resulted in cell death, necrosis, and induction of inflammatory mediators (46, 47). We supposed that necrosis and leakage of hepatocellular contents explained the increased serum TBA levels despite the CYP7A1 and CYP8B1 gene repression in INH-LPS-cotreated animals. Similarly, the combination caused apoptotic hepatocyte death that was already confirmed by a caspase-3 test and TUNEL assay. In contrast to our findings, LPS was found previously to inhibit caspase-3-dependent apoptosis (48), but many previous researchers were in agreement with our findings that LPS potentiated the apoptosis-inducing abilities (49). Together, we could attribute the hepatocyte death to both necrotic and apoptotic actions of the INH-LPS combination.

Our results showed significant reductions in liver antioxidant defense mechanisms, especially GSH, SOD, and T-AOC, while MDA was elevated. In line with these findings, Enriquez-Cortina et al. reported that oxidative stress and inhibition of hepatic antioxidant activities were the key determinants of INH-induced hepatotoxicity (50). Additionally, the hepatic microsomal CYP450 system, mainly CYP2E1, is considered a central role player in the ROS generation pathway, either directly or as a by-product of its metabolic activities through the generation of reactive metabolites (51, 52). As experimentally evident, our obtained results revealed that LPS augmented INH-induced CYP2E1 protein expression levels while repressing CYP2E1 gene expression. This repressive effect on CYP2E1 gene expression could be attributed to LPS-induced inflammation; Abdulla et al. and Hakkola et al. supported this hypothesis (53, 54). Meanwhile, earlier researchers reported CYP2E1 participation in hepatotoxicity by expanding its role in the elevation of INH hepatotoxicity parameters (55, 56). Similarly, CYP2E1 was found to increase liver sensitivity toward LPS and inflammatory mediator toxicity and to potentiate LPS-induced oxidative stress in the liver (57, 58); therefore, both INH and LPS caused overactivation of CYP2E1, which, in return, intensified their toxicity. CYP2E1 involvement in INH toxicity is not limited just to the liver: Shayakhmetova et al. declared that the induction of CYP2E1 in rat testicular tissues treated with INH resulted in the triggering and accumulation of ROS that led to testicular toxicity, DNA fragmentation, spermatogenesis disturbances, and male infertility (59). CYP2E1 also had a functional role in bile acid increment through activation of the bile acid synthesis cascade (56). Furthermore, the effects of CYP2E1 on triglyceride accumulation, apoptotic induction, and hepatic steatosis were previously mentioned and extensively studied (60–62). Hence, it appears that CYP2E1 has a central participation in all INH hepatotoxicity symptoms, including necrosis and steatosis.

Highly active metabolic tissues, including the liver, are characterized by the overexpression of PPARα, which plays different roles as an anti-inflammatory and a key modulator of hepatocyte lipid metabolism (63, 64). In line with this, FXR protects the liver from fat accumulation through potentiation of PPARα-lipid regulatory effects (28). Therefore, the reduction in PPARα activity that occurred due to reduced FXR, inflammation, or INH-LPS cotreatment will promote further inflammation and lipid metabolism disturbances, which explains the elevated level of inflammatory mediators and the accumulation of triglycerides in liver tissues. This reduction, in association with CYP7A1 and CYP8B1 gene repression, explains the elevation of both hepatic TG and TC levels that was experimentally observed in rats and may also provide an explanation for the severe steatosis that appeared in hepatic tissues after INH-LPS cotreatment. Our overall speculations on the hepatotoxicity mechanisms of INH-LPS, as well as the impact of CYP2E1, inflammatory cytokines, and PPARα, are fully illustrated in Fig. 6.

In summary, our results revealed that individual LPS or INH treatment caused minor toxic effects, while cotreatment with INH and LPS provoked INH-induced hepatotoxicity, which was manifested mainly by the elevation of hepatotoxicity biomarkers and lipid metabolic disturbances associated with severe liver necrosis and steatosis. CYP2E1 expression that was fortified by both INH and LPS endotoxins acted as a rate-limiting key in the initiation and propagation of the liver injury. Our study provides clues

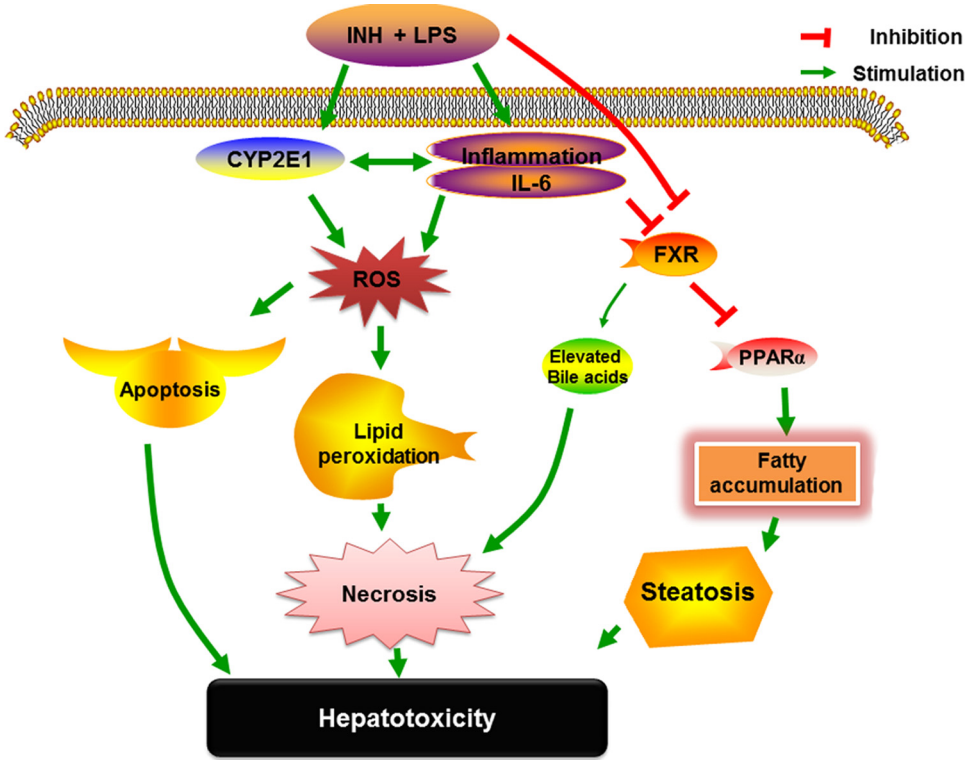

FIG 6 Schematic presentation highlighting the proposed mechanisms by which the INH-LPS combination induces hepatotoxicity.

that may help uncover the multiple concealed mechanisms behind INH hepatotoxicity, for which coadministration of LPS serves as a good model of DILI that could help clinicians understand its pathogenesis.

ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (grants 81273604, 81320108029, and 81573514) and the Natural Science Foundation of Jiangsu Province (BK20151439); this study was partially supported by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD). In addition, this study was supported by the National "Major Scientific and Technological Special Project for Significant New Drugs" project (2015ZX09501004-002-004) and by the Specific Fund for Public Interest Research of Traditional Chinese Medicine, Ministry of Finance (201507004-002).

We declare that there is no conflict of interest.

FUNDING INFORMATION

This work, including the efforts of Luyong Zhang and Zhenzhou Jiang, was funded by National Natural Science Foundation of China (NSFC) (81273604, 81320108029, and 81573514). This work, including the efforts of Zhenzhou Jiang, was funded by Natural Science Foundation of Jiangsu Province (Jiangsu Natural Science Foundation) (BK20151439). This work, including the efforts of Luyong Zhang, was funded by National "Major Scientific and Technological Special Project for Significant New Drugs" project (2015ZX09501004-002-004). This work, including the efforts of Luyong Zhang, was funded by Specific Fund for Public Interest Research of Traditional Chinese Medicine, Ministry of Finance (201507004-002).

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