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Research Paper

Betulinic acid prevention of D-galactosamine/lipopolysaccharide liver toxicity is triggered by activation of Bcl-2 and antioxidant mechanisms

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Abstract

Objectives The hepatoprotective activity and molecular mechanism of betulinic acid (BA) was investigated on acute liver failure induced by D-galactosamine (D-GalN)/lipopolysaccharide (LPS) *in vivo*.

Methods Mice were administered with different doses of BA (20 mg/kg or 50 mg/kg, i.p.) 1 h before injection of D-GalN (700 mg/kg)/LPS (10 μg/kg) and sacrificed 6 h after treatment with D-GalN/LPS.

Key findings Pretreatment with BA significantly prevented the increases of serum aspartate aminotransferase and alanine aminotransferase, while it increased the content of glutathione and catalase, and reduced malondialdehyde. BA showed obvious anti-oxidant effects and prevented D-GalN/LPS-induced apoptosis, as indicated by DNA ladder. BA treatment resulted in regulation of the mitogen-activated protein kinase. We found that BA mediated production of c-jun NH_2-terminal protein kinase and extracellular signal-regulated kinase induced by D-GalN/LPS, promoted the expression of B-cell CLL/lymphoma 2 (Bcl-2) and restored mitochondrial outer membrane permeabilization.

Conclusions The results suggested that BA prevented D-GalN/LPS-induced acute liver failure by upregulation of Bcl-2 and antioxidation and mediation of cytokines causing apoptotic cell death and lessened liver damage.

Keywords antioxidation; bcl-2; betulinic acid; D-galactosamine/lipopolysaccharide; MAPK

Introduction

Betulinic acid (BA) is a lupane-derived triterpenoid, distributed in betula plants and many fruits and vegetables, especially in birch bark. (see Figure 1). BA as an oxidation product of betulin also has been detected in bark extracts.[1] BA exhibits a wide variety of pharmacological and biochemical effects and is considered an effective natural medicine for anti-tumour and anti-HIV therapy. Compared with other medicine, BA possesses powerful targeting properties and less adverse effects. BA and its derivatives are characterized as highly selective inhibitors of cancer and HIV with a novel mechanism of action.[2,3] A major advantage for therapy is that BA could activate metalloprotease and result in cell apoptosis, without any cytotoxicity itself. Pisha[4] has found that BA at a dosage of 500 mg/kg exhibited no detectable toxic side effects. In recent years, research has focused on preparation of BA and its derivatives and their biological activity, synthetic route and key enzyme. In addition to selective anti-tumour and anti-HIV effects, in-vivo and in-vitro studies have demonstrated that some triterpenes effectively reduce the hepatotoxicity induced by carbon tetrachloride, paracetamol (acetaminophen), ethanol and cadmium.[5–10] The underlying mechanisms for these responses are not well understood. Our study focused on an investigation of the hepatoprotective effects and related anti-apoptotic molecular mechanisms of BA on acute liver damage.

Acute liver failure, such as acute viral hepatitis, is a devastating liver disease with a progressive course, which is associated with cell death, inflammation and regeneration. Hepatocyte death mainly results in acute liver damage, with consequences of pro-inflammatory cytokines and oxidative stress. Two pathways of liver cell death exist, namely

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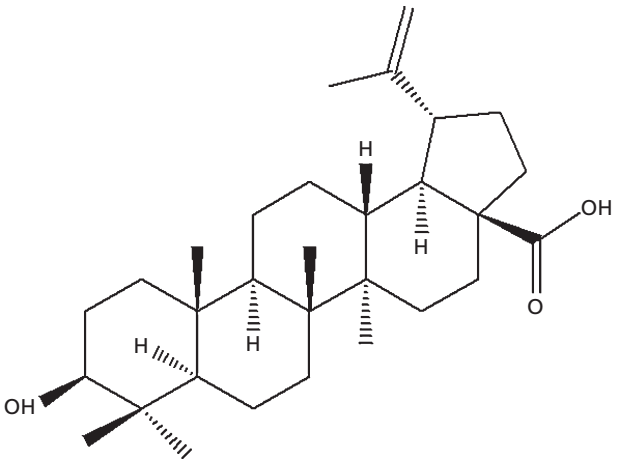

Figure 1 Chemical structure of betulinic acid.

apoptosis and necrosis. Apoptosis is an energy consuming process, which involves caspases, a family of cysteine proteases. Apoptosis is manifest by nuclear and cytoplasmic shrinkage without disturbance of cell membrane integrity or liberation of intracellular content.[11] Apoptosis can be triggered by extrinsic or intrinsic mechanisms, the former involving activation of death receptors located on cell membranes and the latter due to oxidative stress of mitochondria and endoplasmic reticulum. The specific caspases are involved according to the type of proapoptotic stimulus.[12] The other pathway of cell death, necrosis, involves depletion of adenosine triphosphate (ATP), with resultant cell swelling and lysis leading to release of cellular content and secondary inflammation. At a molecular level, apoptosis occurs as a result of the sequential activation of a series of signal transduction pathways, such as the mitogen-activated protein kinase (MAPK) family.[13] In the MAPK family, p38 kinase, extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) are activated by three homologous but distinct signalling pathways. Ras/MAPK and PI3K/Akt signal transduction pathways are important apoptosis-associated signalling pathways.[14] Among these, signalling molecules in Ras/MAPK pathway include Ras, Raf, MEK1/2 and ERK1/2; while major signalling molecules in PI3K/Akt include PI3K, PIP3, PDK1/2 and Akt. Ras/MAPK and PI3K/Akt signalling pathways mutually affect, especially between ERK and Akt. They could affect some downstream apoptosis-associated molecules, such as Bax, Bcl-2, caspase-3 and caspase-7. In this study, injection of D-galactosamine (D-GalN) and lipopolysaccharide (LPS) was used to induce acute liver damage in mice, elucidating the mechanisms of clinical liver complaints and evaluating the efficiency of hepatoprotective activity of various ingredients. D-GalN increases the sensitivity of animals to LPS and augments the lethality rate.[15] The experiments investigated whether BA showed a hepatoprotective effect against D-GalN/LPS-induced acute liver damage, how BA could mediate the expression and function of apoptosis-associated signal pathway, what was the regulation of cytokines causing apoptotic cell death, and how to lessen liver damage.

Materials and Methods

Reagents

Betulinic acid (purity >98%) was obtained from Skyherb Technologies (Hangzhou, China). LPS and D-GalN were obtained from Sigma-Aldrich Biotechnology (St Louis, MO, USA). Caspase-3, Bcl-2, Bax, JNK (p-) JNK (p-) ERK, α-tubulin antibodies and the peroxide-conjugated secondary antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). DAB staining kit was purchased from Solarbio Inc. (Beijing, China). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), malondialdehyde (MDA), glutathione (GSH) and catalase (CAT) assay kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Animals

Healthy male C57BL/6 mice, 6–7 weeks old, 22–26 g, were purchased from Experimental Animal Centre, Jilin University (Changchun, China; license number, SYXK (JiLin Province) 2002–0002). All mice were acclimatized to the laboratory environment for one week before the experiment. Mice were allowed free access to drinking water and food under constant room temperature (22 ± 2°C) and humidity (50 ± 10%) conditions with an automatic 12-h light–dark cycle. Rodents were handled according to SOT (Society of Toxicology) Guiding Principles in the Use of Animals in Toxicology (Revised, 1999). The animal care committee of the local institution approved the study.

Treatments

BA was studied in a variety of experimental animals *in vivo* at a dose range of 10–150 mg/kg, depending on species and route of administration. In this study, dose ranges and sacrifice time of mice were selected based on previous research and a preliminary study. Mice were fasted overnight (16–18 h) before administration of a single intraperitoneal dose of D-GalN (700 mg/kg) and LPS (10 μg/kg) dissolved in sterile saline (pH 7.4). Mice were randomly divided into five groups: normal group, BA50 single group, D-GalN/LPS group, BA20 group and BA50 group. The normal group was administered with an equal volume saline, and the BA50 single group with BA at a dose of 50 mg/kg only. The BA20 group and BA50 group were administered with BA 20 or 50 mg/kg 1 h before D-GalN/LPS injection. Except for the normal and BA50 single groups, the other groups were treated with D-GalN/LPS and sacrificed at 6 h after D-GalN/LPS injection.

Determination of biochemical parameters

At 6 h after D-GalN/LPS treatment, mice were sacrificed, and the blood samples were collected and then centrifuged at 3000*g* for 15 min at 4°C, and serum ALT, and AST levels were measured using a microplate reader (Tecan, Männedorf, Switzerland). Mice were killed at 6 h after D-GalN/LPS injection and the same liver lobe excised from each mouse. Tissue samples were kept at −80°C for subsequent analysis. The frozen liver slices were washed in ice-cold EDTA solution (0.02 mol/l), blotted, dissected to remove connective tissue, weighed and then homogenised with saline. GSH, MDA and

CAT activity was measured according to the manufacturers' instructions. Liver protein was determined using Coomassie Brilliant Blue. The GSH kit detects GSH activity through yellow tetramethyl-benzidine and oxidised glutathione produced by the combination of GSH and dithio-nitrobenzene. The MDA assay kit detects MDA activity by measuring a red product formed when MDA reacts with thiobarbituric acid. CAT can catalyse oxidation of H_2O_2 to form a red chromogenic substrate that is detected by the CAT kit.

Western blot analysis

Six hours after injection of D-GalN/LPS, the mice were sacrificed to collect blood from arteria carotis communis, the livers from the five groups of mice were obtained and frozen at −80°C until use. The frozen tissues were homogenized in lysis buffer (50 mM Tris-HCl, 10 mM EDTA, 150 mM NaCl, pH 7.4) containing 1 mM PMSF (phenylmethanesulfonyl fluoride), 1 μg/ml leupeptin and 0.5 μg/ml pepstatin at 4°C. After centrifugation for 30 min at 12 000*g* (4°C), the protein concentration in the supernatant was determined using a BAC protein assay kit (Pierce, Rockford, IL, USA). Lysates (50 μg) were electroblotted onto a nitrocellulose membrane following electrophoretic separation on a 10% or 12% SDS-polyacrylamide gel. Blotted membranes were incubated for 1 h with blocking solution (1% PBS/Tween 20 PBST, Phosphate Buffer Saline Tween) containing 5% skimmed milk (w/v) at room temperature, followed by incubation overnight at 4°C with 1 : 500 dilution of caspase-3, Bcl-2, Bax, ERK, p-ERK, JNK, p-JNK, α-tubulin primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Membranes were washed four times with 1% PBST and incubated with 1 : 2000 dilution of horseradish peroxidase-conjugated goat anti-rabbit or rabbit anti-mouse IgG secondary antibody for 1 h at room temperature. Membranes were washed six times in PBST and then developed by ECL (Beyotime, Jiangsu, China). The scan densitometric analysis was carried out with a GDS-8000 UVP photoscanner and LAB WOEK45 image software (Bio-rad).

DNA fragmentation electrophoresis analysis

Genomic DNA was extracted from liver tissue using a Wizard genomic DNA purification kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. Approximately 50 μg of DNA were loaded into each well and 1.5% agarose gel electrophoresis carried out at 100 V in a TBE buffer (Tris-borate-EDTA) for 30 min. After electrophoresis, DNA was visualized by soaking the gel in the TBE buffer containing 0.1 μg/ml ethidium bromide. The gel was observed by using UV light and photographed.

Statistical analysis

Data are expressed as mean values ± SEM and comparisons of data was done by one-way analysis of variance followed by the Tukey's multiple comparison tests. Survival rate was analysed with Log linear model or Mantel-Cox test. Statistically significant differences between groups were defined as $P < 0.05$. Calculations were performed with the GraphPad Prism program (Graphpad Software Inc., San Diego, CA, USA).

Results

Effect of betulinic acid on the survival of mice treated with D-GalN/LPS

To assess the beneficial effect of BA on acute liver injury induced by D-GalN/LPS in mice, survival studies were performed. As shown in Figure 2, D-GalN/LPS group mice died, beginning at 10 h, and the survival rate was 22.22% (2/9 mice survived) at 24 h after D-GalN/LPS injection. Injection of BA before D-GalN/LPS administration significantly increased the survival rate of mice within 24 h. After pretreatment with BA (50 mg/kg, i.p.), the survival rate was 100% (all mice survived). BA treatment, 20 mg/kg intraperitoneally, elevated the survival rate to 83.33% after D-GalN/LPS injection. BA administration by oral gavage, 20 and 50 mg/kg, still offered 30.0% (3/10 mice survived) and 54.54% (6/11 mice survived) protection from liver injury, respectively. The results indicated that BA treatment can invariably increase the survival of mice injected with D-GalN/LPS, and intraperitoneal injection was better than oral gavage.

Effect of betulinic acid on serum alanine aminotransferase and aspartate aminotransferase levels

The protective effects of BA on the D-GalN/LPS induced serum ALT and AST levels are shown in Figure 3. The administration of D-GalN/LPS markedly increased serum ALT and AST levels, which reached 1157.0 ± 259.6 IU/l ($P < 0.001$) and 522.1.9 ± 69.3 IU/l ($P < 0.001$), respectively, while the normal group of mice had levels of 43.33 ± 10.8 IU/l and 31.89 ± 0.63 IU/l, respectively. However, pretreatment with BA reduced both serum ALT and AST levels. Serum ALT levels were significantly decreased by 86.7% ($P < 0.001$) in the BA20 + D-GalN/LPS group and by 97.7% ($P < 0.001$) in the BA50 + D-GalN/LPS group compared with D-GalN/LPS. Serum AST levels were also decreased by 48.7% ($P < 0.05$) in

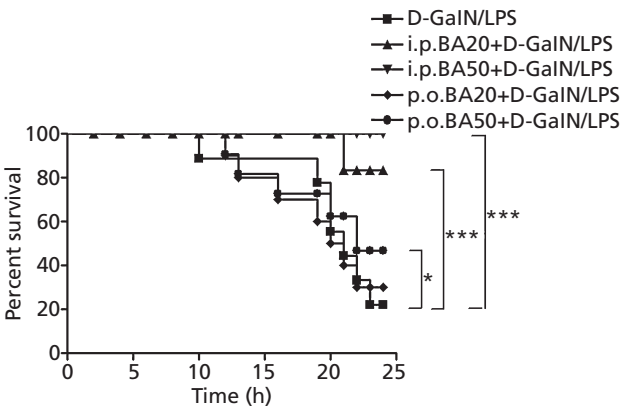

Figure 2 Effect of betulinic acid treatment on D-GalN/LPS-induced survival rate in mice. The mice were given BA (20, 50 mg/kg) by oral gavage (p.o.) or intraperitoneal injection (i.p.) 1 h before injection of D-GalN/LPS. Survival rate of all mice was monitored within 24 h. $n = 9–11$ for each group, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with D-GalN/LPS group.

the BA20 + D-GalN/LPS group and by 71.3% ($P < 0.01$) in the BA50 + D-GalN/LPS group compared with D-GalN/LPS.

Effect of betulinic acid on hepatic glutathione, malondialdehyde and catalase levels

Six hours after D-GalN/LPS administration, the D-GalN/LPS group had significantly decreased GSH concentration, by 66.3% ($P < 0.01$) compared with the normal group. However, pretreatment with BA significantly recovered the D-GalN/LPS-induced GSH depletion to 783.4 ± 80.5 mg/g protein ($P < 0.05$) and 648.0 ± 95.9 mg/g protein, respectively, in the D-GalN/LPS + BA20 group and D-GalN/LPS + BA50 group (Figure 4a). The concentration of hepatic MDA was significantly increased in the D-GalN/LPS group (14.7 ± 2.0 nmol/mg protein, $P < 0.05$) compared with the normal group (7.9 ± 1.2 nmol/mg protein). However, pretreatment with BA brought about significant decrease in MDA levels ($P < 0.001$, $P < 0.001$) (Figure 4b). In the D-GalN/LPS group, the CAT content at 6 h after injection was significantly lower compared with the normal group ($P < 0.001$) and BA50 only group ($P < 0.05$). Pretreatment with different doses of BA significantly increased the levels of CAT in the serum of mice challenged with D-GalN/LPS ($P < 0.001$, $P < 0.01$) (Figure 4c).

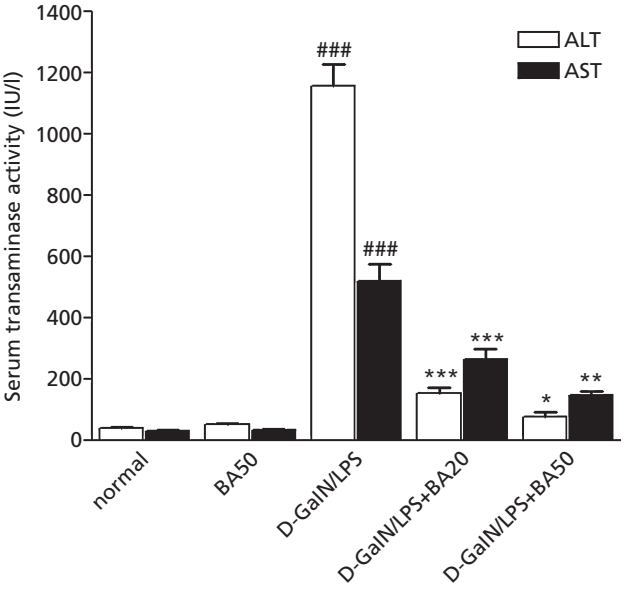

Figure 3 Effect of betulinic acid on serum alanine aminotransferase (ALT) and aspartate aminotransferase levels (AST) in mice. The mice were given BA (20, 50 mg/kg, i.p.) 1 h before injection of D-GalN/LPS. Serum ALT and AST were determined 6 h after D-GalN/LPS intoxication. Bar represents mean ± SEM., $n = 10$ mice per group, ###$P < 0.001$ vs normal or BA50 only; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs D-GalN/LPS.

Effect of betulinic acid on caspase-3, and Bcl-2 family protein expressions

To further investigate the mechanism of BA on D-GalN/LPS-induced liver injury, we investigated caspase-3 and Bcl-2 family protein expressions involved in the D-GalN/LPS-induced apoptosis. As shown in Figure 5, strong increases in the levels of activated caspase-3 resulted from D-GalN/LPS administration. However, pretreatment with BA (20 mg/kg or 50 mg/kg) decreased caspase-3 activity induced by D-GalN/LPS, and active caspase-3 expression with BA 50 mg/kg significantly decreased compared with D-GalN/LPS (Figure 5b). The Bcl-2 family proteins function to either inhibit (Bcl-2) or promote (Bax) apoptosis. Pretreatment with BA showed less effect on Bax expression. Bcl-2 expression was significantly decreased in the D-GalN/LPS group compared with the normal group, while such decrements were altered by BA, and Bcl-2 expression markedly increased in the BA-pretreatment

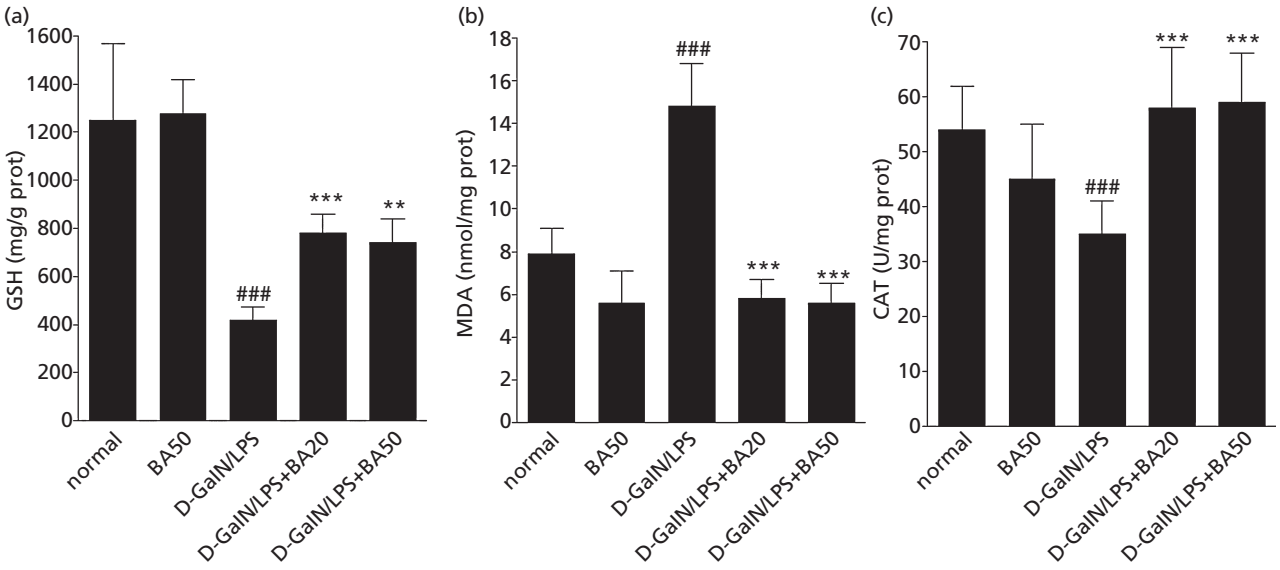

Figure 4 Effect of betulinic acid on liver glutathione (GSH), malondialdehyde (MDA) and catalase (CAT) levels in mice. The mice were given BA (20, 50 mg/kg, i.p.) 1 h before injection of D-GalN/LPS. Liver GSH, MDA and CAT levels were determined 6 h after D-GalN/LPS intoxication. Bar represents mean ± SEM., $n = 10$ mice per group, ###$P < 0.001$ vs normal or BA50 only; **$P < 0.01$, ***$P < 0.001$ vs D-GalN/LPS.

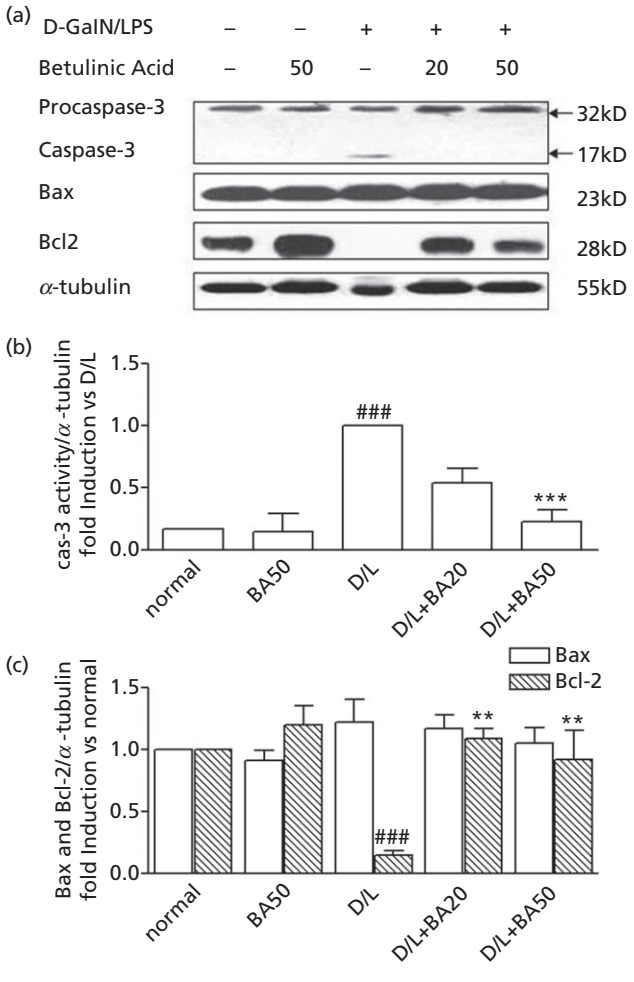

Figure 5 Effect of betulinic acid pretreatment on caspase-3 and Bcl-2 family protein in mice treated with D-GalN/LPS. The mice were given BA (20, 50 mg/kg, i.p.) 1 h before injection of D-GalN/LPS. Six hours after D-GalN/LPS intoxication, caspase-3 and Bcl-2 family protein expression in liver were detected using Western blotting. Equal loading of proteins was verified by α-tubulin blotting. ###$P < 0.001$ vs normal; **$P < 0.01$, ***$P < 0.001$ vs D-GalN/LPS.

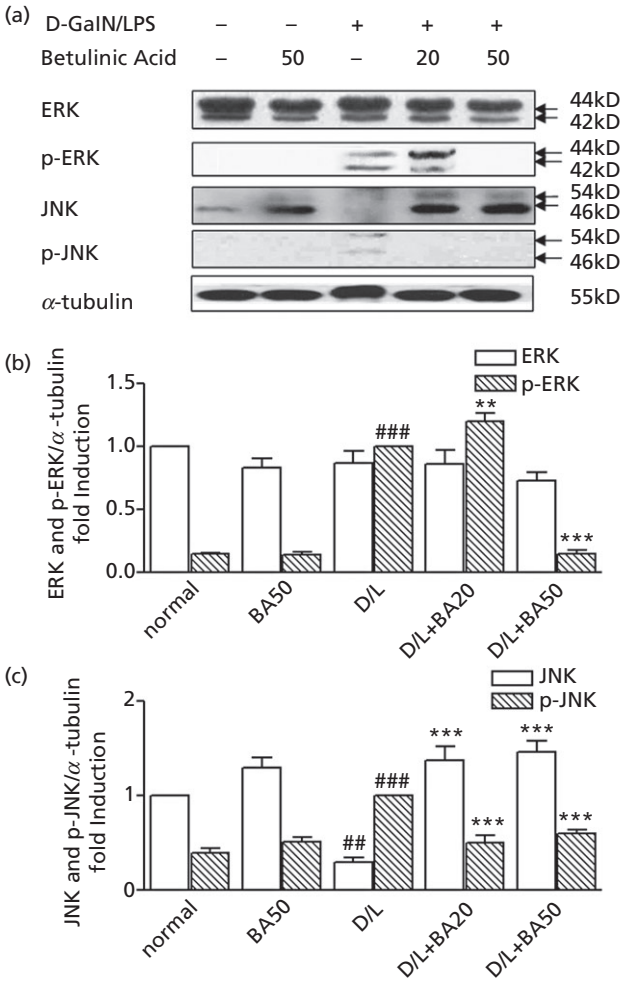

Figure 6 Effect of betulinic acid pretreatment on MAPK protein in mice treated with D-GalN/LPS. The mice were given BA (20, 50 mg/kg, i.p.) 1 h before injection of D-GalN/LPS. 6 h after D-GalN/LPS intoxication, JNK protein expression in liver was detected using Western blotting. Equal loading of proteins was verified by α-tubulin blotting. ##$P < 0.01$, ###$P < 0.001$ vs normal; **$P < 0.01$, ***$P < 0.001$ vs D-GalN/LPS.

groups (Figure 5c). Therefore, BA could prevent Bcl-2 depletion induced by D-GalN/LPS, but had little regulatory effect on Bax.

Effect of betulinic acid on mitogen-activated protein kinase expression

To confirm whether MAPK activation was inhibited in the liver of BA-treated mice after D-GalN/LPS administration, phosphorylation of JNK and ERK, a major downstream target of JNK, was examined by Western blot analysis (Figure 6a). An increase of immunoreactivity was seen in the D-GalN/LPS group for phosphorylated JNK (p-JNK) and ERK (p-ERK) after D-GalN/LPS administration, which indicated that phosphorylation of JNK and ERK increased in the D-GalN/LPS group (Figure 6b and 6c). Nevertheless, pretreatment with BA significantly decreased the D-GalN/LPS-induced p-JNK and p-ERK protein expressions (except for BA 20 mg/kg on p-ERK expression). However, Western blotting results demonstrated that BA blocked phosphorylation of JNK and ERK.

Apoptosis inhibition of betulinic acid in mice treated with D-GalN/LPS

The main character of apoptosis is the cleavage of nuclear DNA into multiple fragments. DNA from treated and non-treated BA was examined using 1.5% agarose gel electrophoresis (Figure 7). Genomic DNA fragmentation was observed in the livers of mice treated with D-GalN/LPS alone 6 h after D-GalN/LPS injection, while almost no DNA fragmentation was observed in the livers of mice pretreated with 20 or 50 mg/kg BA. These data showed that DNA fragmentation occurred in mice treated with D-GalN/LPS, and that DNA fragmentation was effectively prevented by BA pretreatment.

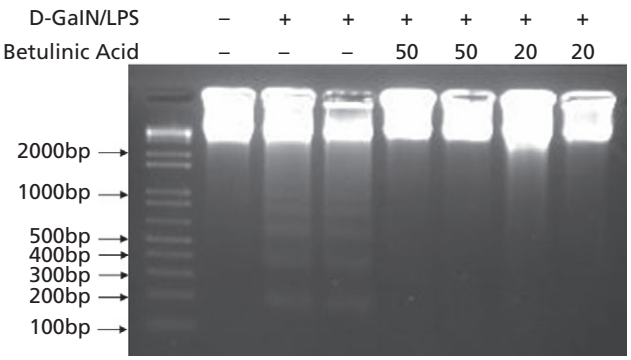

Figure 7 Apoptosis inhibition by betulinic acid in mice treated with D-GalN/LPS. Mice were adiministered with the indicated concentrations of BA 1 h before D-GalN/LPS injection, then the DNA fragmentation was analysed by gel electrophoresis as described in Methods. Lane 1, DNA marker; lane 2, normal group; lane 3, 4, D-GalN/LPS group; lane5, 6, BA (50 mg/kg) pretreatment groups; lane 7, 8, BA (20 mg/kg) pretreatment groups.

Discussion

In this study, we have proved the hepatoprotective effect of betulinic acid on D-GalN/LPS-induced acute liver damage in mice. BA pretreatment improved the survival rate of mice administered with D-GalN/LPS, and attenuated serum transaminase, which showed dose-dependent reduction in enzyme activity. Mice treated with D-GalN/LPS presented significant decreases in liver levels of enzymatic and non-enzymatic antioxidants and increases of lipid peroxidation compared with the normal group. D-GalN/LPS caused accumulation of MDA and depletion of GSH and CAT. BA administration increased GSH and CAT activity, and decreased MDA level, which meant one of the hepatoprotective mechanisms of BA might be via the antioxidant defence system. As a proof, the activity of enzymatic antioxidants in the mice pretreated with BA were significantly increased compared with the D-GalN/LPS group. These antioxidant enzymes play an important role in the body defence mechanism against the harmful effects of active oxygen species and free radicals in biological systems. Consequently, oxidative stress results in apoptosis and cell damage, and it could directly break the balance of the intracellular redox state or indirectly activate the cellular signal transduction pathway.[16] Furthermore, we also used DNA gel electrophoresis to confirm that BA decreased apoptosis caused by D-GalN/LPS.

Apoptosis is another characteristic of acute liver damage caused by D-GalN, followed by cytoplasmic shrinkage, nuclear condensation and DNA fragmentation.[17,18] Apoptosis can be induced through both extrinsic and intrinsic pathways. The former involves the ligation of the TNF/Fas-receptor, followed by caspase-8 activation, which in turn either directly activates caspase-3 or merges by the mitochondrial pathway via the triggering of the Bcl-2 family member, p22/bid. The latter involves the mitochondria-mediated caspase-9 activation pathway. Both of these pathways, though, converge in the activation of caspase-3, and ultimately lead to cell death.[19–22] However, we found that BA could attenuate the apoptosis in liver through the molecular mechanisms of caspase-3 activation and Bcl-2 family. Apoptosis is a regulated form of cell demise that can be induced or blocked by groups of specific stimuli. Bcl-2 family member proteins appear to perform an important regulatory function of cell survival and cell death via the blockage of triggering of both the death-receptor and mitochondrial apoptosis pathways.[23–25] Two different types of Bcl-2 family proteins regulate the triggering of apoptosis. Bcl-2 is an antiapoptotic protein whereas Bax is a proapoptotic protein. We observed that D-GalN/LPS induced the up-regulation of Bax and the down-regulation of Bcl-2. However, BA pretreatment could significantly inhibit the down-regulation of Bcl-2, while having little effect on Bax expression. These results further indicate that the protective effect of BA against D-GalN/LPS is associated with inhibition of upstream of the mitochondrial apoptosis pathway.

MAPK cascades are involved in the signalling of various cellular responses, and are the terminal enzymes in the three-kinase cascade. The MAPK family includes ERK-1/2, JNK-1/2 and p38 MAPK, which are involved in cell death, proliferation and inflammation. The MAPK pathway is activated through a cascade of sequential phosphorylation. JNK modulates some Bcl-2 family proteins on multiple levels: transcriptional, post-transcriptional and post-translational.[26–29] The ERK1/2 cascade is activated through receptor-mediated signalling stimuli and is associated with cell proliferation differentiation and survival. In our study, we found that BA inhibited ERK and JNK phosphorylation through a mitochondria-dependent mechanism, and inhibited migration through activation of the ERK and JNK pathways. We also detected expression of p38 protein by Western blotting, but no significant alteration between D-GalN/LPS and BA administration, which indicated that BA mediated ERK and JNK, without referring to p38 (data not shown here). The experiment suggested that BA could decrease the phosphorylation level of JNK and ERK.

In this study, to clarify the actions of BA on apoptosis-associated signalling pathways in D-GalN/LPS-induced mice, we investigated the anti-oxidant and anti-apoptosis effects of BA and the roles of MAPKs, intracellular ROS and caspases. Our results indicated that BA showed a potent hepatoprotective effect on D-GalN/LPS-induced acute liver damage in mice. The primary mechanisms underlying the protective effects of BA might be due to its inhibition of lipid peroxidation and alleviation of GSH depletion, possibly involving both exterior and internal pathways. In the exterior pathway, BA interferes with MAPK through the activation of ERK and JNK. Activation of ERK and JNK causes mitochondrial outer membrane permeabilization, which refers to the Bcl-2 family. BA promotes the expression of Bcl-2 and supplies anti-apoptosis power. However, further investigation is required to determine the exact protective mechanism. Therefore, it is worth investigating the role of BA on apoptosis mechanisms in *in vivo* and *in vitro* conditions that include apoptosis cytokines.

Conclusions

BA prevented D-GalN/LPS-induced acute liver failure by upregulation of Bcl-2 and antioxidation; mediation of cytokines caused apoptotic cell death and lessened liver damage.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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