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Research Paper

Protective effect of JBP485 on concanavalin A-induced liver injury in mice

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Abstract

Objectives Cyclo-*trans*-4-L-hydroxyprolyl-L-serine (JBP485) was first isolated from Laennec (hydrolysate of human placenta). We thought it valuable to clarify the anti-hepatitis molecular mechanism of JBP485 to develop a new oral anti-hepatitis drug.

Methods We investigated the hepatoprotective effect of JBP485 on immune-mediated, concanavalin A (Con A)-induced liver injury in mice. Mice were administered JBP485 before and after injection of Con A (10 mg/kg). Eight hours after Con A, the cytosolic enzyme activity (alanine aminotransferase, lactate dehydrogenase) in serum, and the enzyme activity or concentration (superoxide dismutase, maleic dialdehyde, myeloperoxidase, nitric oxide) in liver homogenate were determined. The liver slices were investigated to observe changes in histology. The effect of JBP485 on level of tumour necrosis factor-α (TNF-α) and intercellular adhesion molecule-1 (ICAM-1) in liver were detected by immunohistochemistry. Hepatocyte DNA fragmentation was assayed by agarose gel electrophoresis and the transcription of the genes bax and bcl-2 in hepatocytes was determined by reverse transcription-polymerase chain reaction.

Key findings Con A increased the cytosolic and liver homogenate enzyme activity, and the concentrations of ICAM-1 and TNF-α, which were significantly inhibited by JBP485 administration. Also, the increase in DNA fragmentation and decrease in bcl-2/bax mRNA induced by Con A administration were significantly inhibited by JBP485.

Conclusions These results indicated that immune-mediated liver damage can be prevented by JBP485, and that this is mainly associated with immunomodulatory effects on T cells and adhesion molecules, antioxidation, and inhibition of apoptosis.

Keywords apoptosis; concanavalin A; hepatitis; JBP485; Laennec

Introduction

Human placental extract (HPE) has been used to treat a number of liver diseases, including hepatitis and cirrhosis.[1] Laennec (a trade name for hydrolysate of human placenta) is produced by Japan Bioproducts Industry Co. Ltd (Tokyo, Japan) by purification of human placental extracts, and it has been used clinically to treat chronic hepatic injury for over 40 years in Japan.[2] JBP485 (Figure 1), a dipeptide, was one of the constituents isolated from Laennec, and it has been synthesized enantioselectively by chemical means. From our preliminary experiment, we had shown that JBP485 exhibited excellent gastrointestinal absorption[2] and had anti-hepatitis activity in concanavalin A (Con A)-induced hepatocyte toxicity.[3] This suggested that JBP485 was recognized by the peptide transporter system in the gastrointestinal tract. Therefore, it is valuable to clarify the anti-hepatitis molecular mechanism of JBP485 to develop a new oral anti-hepatitis drug.

A well-described mouse model of T-cell-dependent liver injury is inducible by injection of the T-cell mitogenic plant lectin concanavalin Con A, which leads to fulminant hepatitis within eight hours.[4] The liver injury model induced by Con A injection into mice is regarded as an appropriate model for studying immune-mediated liver diseases.

In this study, to clarify its anti-hepatitis molecular mechanism we examined the protective effect of JBP485 on immune-mediated liver injury in mice.

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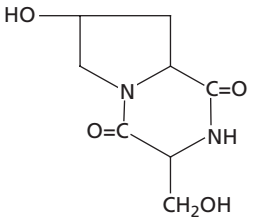

Figure 1 Chemical structure of JBP485

Materials and Methods

Reagents

JBP485 was obtained from Japan Bioproducts Industry Co. Ltd (Tokyo, Japan). Con A and collagenase were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Anti-mouse tumour necrosis factor-α (TNF-α) antibody and anti-mouse intercellular adhesion molecule-1 (ICAM-1) antibody were purchased from Boster Biotechnology Co. Ltd (Wuhan, China). DNA fragmentation and reverse transcription-polymerase chain reaction (RT-PCR) reagents were obtained from TaKaRa Biotechnology (Dalian) Co. Ltd (Dalian, China). Other reagents will be further specified.

Animals

Female BALB/c mice, 20–24 g (Experimental Animal Center of Dalian Medical University, Dalian, China), were housed at 23 ± 2°C under a 12-h light–dark cycle, at 50 ± 5% relative humidity throughout the whole experimental period. All mice were allowed free access to water and chow diet. All experimental protocols were approved by the Institutional Animal Ethics Committee of Dalian Medical University, which follows the standards of the Committee for the Purpose of Control and Supervision of Experimentation on Animals, Government of China.

Concanavalin A-induced hepatitis

Mice were divided randomly into three groups: the Con A group, the Con A + JBP485 group and the normal group. In the Con A group and Con A + JBP485 group, mouse liver damage was induced by injection of Con A (10 mg/kg, 1.5 mg/ml),[5,6] dissolved in pyrogen-free saline, through the tail vein. JBP485 (25 mg/kg, 2.5 mg/ml) dissolved in pyrogen-free saline was administered orally by gavage to mice 0.5 h before and 2 and 5 h after challenge with Con A in the Con A + JBP485 group. The Con A group was given the same volume of pyrogen-free saline without JBP485. The normal group was given the same volume of pyrogen-free saline instead of Con A and JBP485 at the same times.

Analysis of serum transaminase activity and determination of superoxide dismutase, maleic dialdehyde, myeloperoxidase and nitrite in liver tissue

Injection of Con A leads to fulminant hepatitis within 8 h.[4] Therefore, hepatocyte damage was assessed 8 h after Con A administration by measuring the serum enzyme activity of alanine aminotransferase (ALT) and lactate dehydrogenase (LDH)[7–9] using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Mouse liver was removed immediately after blood was harvested and was kept at –20°C. Liver samples were thawed, weighed and homogenized on ice with phosphate-buffered saline (pH 7.4). The homogenate was used for assays of superoxide dismutase (SOD), maleic dialdehyde (MDA), myeloperoxidase (MPO) and nitrite. The assays were carried out using assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and performed exactly as described by the manufacturer's instructions. Protein was determined using the Lowry method.

Histology and immunohistochemistry

Mouse liver was removed 8 h after Con A injection, fixed in 10% phosphate-buffered formaldehyde, embedded in paraffin and then sectioned (4 μm) and stained with haematoxylin–eosin (H&E) for morphological examination. For immunohistochemistry studies, sections were incubated overnight at 4°C with 1 : 100 and 1 : 150 dilutions of anti-mouse TNF-α and ICAM-1, respectively. Antibody binding was visualized using biotinylated rabbit anti-goat IgG, avidin–biotin complex and 3.3′-diaminobenzidine. In the immunohistochemistry experiment, sections in each group were photographed by an OLYMPUS BX51 TR-32000 (Japan) automatic photomicrography system, using Imagepro-plus 4.5 (USA) 13 × 517 R image analysis software.

RT-PCR analysis of expression of apoptosis-related genes, bax and bcl-2, in hepatocytes

Total cytoplasmic RNA was extracted from 6.0×10^6 to 7.0×10^6 hepatocytes in each group using the TRIzol Reagent (Invitrogen, Beijing, China) according to the manufacturer's instructions. Two-step RT-PCR was performed according to the protocol of the kit (Takara, Dalian, China) and amplified in a GeneAmp PCR system (Techne TC512, UK). The concentration of RNA was determined by absorbance at 260 nm, and its integrity was confirmed by means of electrophoresis on 1% agarose gels, and then stained with 0.1 mg/l ethidium bromide. One microgram of total RNA for each sample was subjected to RT-PCR. The following oligonucleotide pairs were used: reverse (5′-CGCCGGGCTGGGGATGACTTCT-3′) and forward primer (5′-CACTTGTGGCCCAGGTATGC-3′) for bcl-2, and reverse (5′-GAGCAGCCGCCCCAGGATG-3′) and forward primer (5′-GGTGAGCGAGGCGGTGAGGAC-3′) for bax. To verify that equal amounts of cDNA were presented in the PCR reaction, β-actin with the reverse (5′-GGGCACAG-TGTGGGTGAC-3′) and forward primer (5′-CTGGCACCA-CACCTTCTAC-3′) was used as an internal control. For bcl-2, the RT-PCR conditions were as follows: reverse transcription at 42°C for 30 min, inactivation of the reverse transcriptase at 99°C for 5 min and 4°C for 5 min, predenaturation at 94°C for 2 min, 30 cycles at 94°C for 30 s (denaturation), 61°C for 30 s (annealing), 72°C for 1 min (elongation) and, finally, one cycle at 72°C for 7 min. For bax RT-PCR, the protocol was similar, but for β-actin, the temperature used was 53°C for annealing. The expected fragment lengths were 428 bp for bcl-2, 417 bp for bax and

364 bp for β-actin. The products were electrophoresed on an agarose gel and visualized under UV light. Semiquantitative evaluation was performed using the UVP Bio Spectrum Imaging System (Ultra-Violet Products Ltd, Cambridge, UK). Levels of bcl-2 and bax mRNA were calculated in arbitrary units as the proportion of PCR product intensity to β-actin PCR product intensity from the same RNA sample.

Statistical analysis

Statistical analysis was performed using SPSS (version 9; SPSS, Chicago, USA). All data are expressed as the mean ± SEM of n separate experiments. Groups of data were compared with analysis of variance followed by Student's t-test (one-tailed). $P < 0.05$ was regarded as significant.

Results

Decrease in activity of serum enzymes after oral administration of JBP485 in concanavalin A-intoxicated mice

To examine whether JBP485 promotes the repair of liver injury, we determined the change in the activity of enzymes such as ALT and LDH in serum from Con A-intoxicated mice after administration of JBP485. The increase in the activity of these biochemical markers for liver injury in the serum caused by Con A intoxication was inhibited by oral administration of JBP485 at a dose of 25 mg/kg (2.5 mg/ml) (Figure 2a, b).

Antioxidative effect of JBP485 in liver tissue of concanavalin A-intoxicated mice

To examine the antioxidative effect of JBP485 on Con A-induced hepatitis, we analysed the SOD activity and MDA production in liver tissue. Compared with the normal group, the level of liver SOD in the Con A group was reduced by 33.9% (92.3 ± 1.9 U/mg protein vs 61.0 ± 2.1 U/mg protein, $P < 0.01$). However, after administration of JBP485, the liver SOD activity was replenished to 81.4 ± 1.4 U/mg protein, which was an increase of 33.4% versus the Con A group ($P < 0.01$) (Figure 2c). By contrast, there was an increase in the MDA level in liver homogenates 8 h after Con A injection (2.2 ± 0.05 nmol/g protein in normal mice vs 3.6 ± 0.07 nmol/g protein in the Con A alone group,

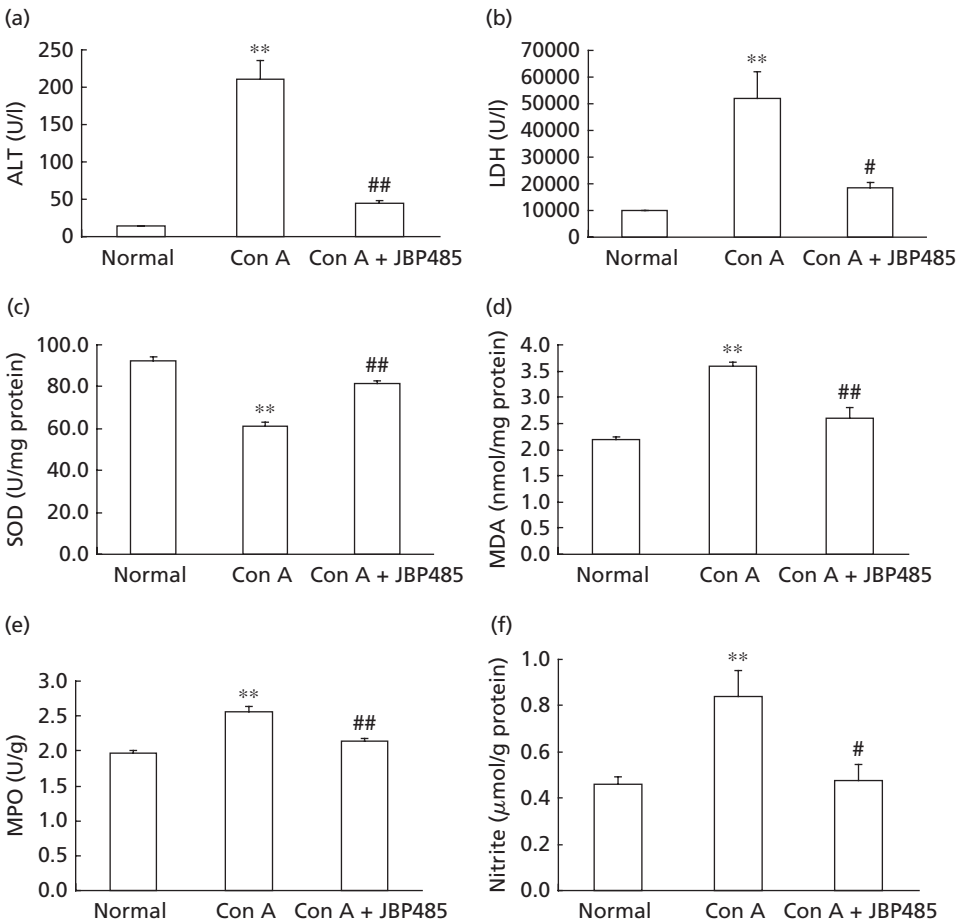

Figure 2 Effect of JBP485 on markers of liver damage in mice. The effect of JBP485 (25 mg/kg) on serum alanine aminotransferase (ALT) (a), lactate dehydrogenase (LDH) (b), superoxide dismutase (SOD) (c), maleic dialdehyde (MDA) (d), myeloperoxidase (MPO) (e) and nitrite (f) levels in BALB/c mice was determined 8 h after intravenous injection of concanavalin A (Con A) (10 mg/kg). Data are expressed as mean ± SEM, $n = 8$ per group for ALT and LDH; $n = 6$ per group for others. $^{**}P < 0.01$ vs normal group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs Con A group.

$P < 0.01$). After administration of JBP485, the MDA level was reduced by 72.2% (2.6 ± 0.19 nmol/g protein, $P < 0.01$) vs the Con A group (Figure 2d).

Effect of JBP485 on myeloperoxidase activity and nitrite content in liver tissue of concanavalin A-intoxicated mice

To examine the effect of JBP485 on Con A-induced liver injury in response to hepatic neutrophil recruitment and nitric oxide (NO) expression, the MPO content and the levels of nitrite in liver tissue were measured, respectively, after oral administration of JBP485 in Con A-treated mice. MPO is an enzyme restricted mainly to neutrophils, which reflects neutrophil tissue infiltration. The levels of nitrite reflect the level of NO in liver tissue indirectly. There was a marked increase in liver MPO content in the Con A group compared with the normal group (2.57 ± 0.06 U/g vs 1.97 ± 0.03 U/g, $P < 0.01$). However, after administration of JBP485 at a dose of 25 mg/kg, there was a reduction in MPO content compared with the Con A group (2.14 ± 0.04 U/g vs 2.57 ± 0.06 U/g, $P < 0.01$) (Figure 2e). There was a significant increase in nitrite levels (183.04%) in the Con A group compared with the normal group (0.842 ± 0.111 μmol/g protein vs 0.460 ± 0.032 μmol/g protein, $P < 0.01$). JBP485 resulted in a marked reduction in nitrite content compared with the Con A group (0.478 ± 0.067 μmol/g protein vs 0.842 ± 0.111 μmol/g protein, $P < 0.05$), to almost the normal level (Figure 2f).

Effect of JBP485 on the histological changes in the liver of concanavalin A-intoxicated mice

To evaluate the effect of JBP485 on hepatitis, we examined the histological changes in the liver of mice at 8 h after Con A injection. Compared with normal cells (Fiure 3a, d), the portal areas and hepatic lobuli were infiltrated with mononuclear and polymorphonuclear cells. Massive necrosis with cytoplasmic swelling of most hepatocytes was observed in Con A-treated mice by microscopic examination (Figure 3b, e). However, the inflammatory infiltration, necrosis and cytoplasmic swelling decreased in JBP485-treated mice (Figure 3c, f).

Immunohistochemical analysis for liver TNF-α and ICAM-1 in concanavalin A-intoxicated mice

To determine whether JBP485 can affect cytokine and adhesion molecule expression by macrophages and hepatocytes, we examined the effect of JBP485 on the expression of TNF-α and ICAM-1 in liver tissue of Con A-treated mice

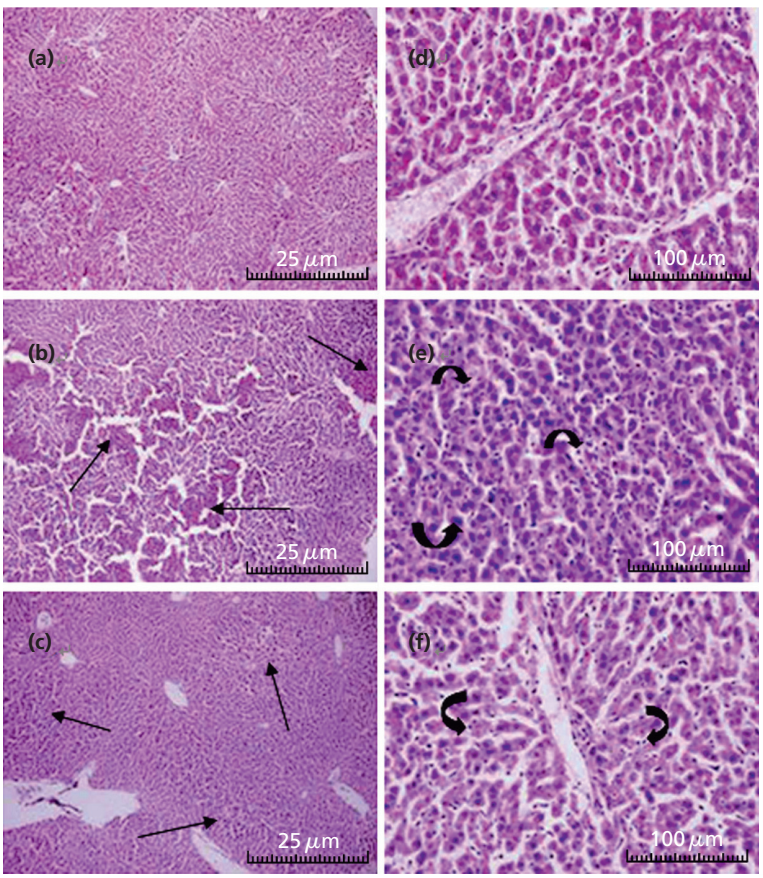

Figure 3 Effect of JBP485 on hepatocyte necrosis and inflammatory infiltration in mice. The effect of JBP485 on the change in hepatocyte necrosis (straight arrow) and inflammatory infiltration (curved arrow) was determined 8 h after concanavalin A (Con A)-induced liver injury in BALB/c mice. (a, d) Liver section from normal group. (b, e) Liver section from mouse 8 h after Con A injection. (c, f) Liver section from mouse treated with JBP485 (25 mg/kg). Inflammation and necrosis were markedly alleviated compared with the Con A group. ($n = 6$ per group; Hematoxylin-eosin (H&E), magnification × 100 and × 400, respectively).

after oral administration of JBP485. The expression of TNF-α and ICAM-1 in liver tissue was examined by immunohistochemistry (Figure 4). In the normal group (Figure 4c, f), the expression of TNF-α and ICAM-1 was visualized as a light brown colour following immunostaining. The expression of these entities was highly upregulated 8 h after Con A administration (Figure 4a, d). Compared with the Con A group, the increase in TNF-α and ICAM-1 expression was significantly suppressed by oral administration of JBP485 (Figure 4b, e).

Effect of JBP485 on concanavalin A-induced DNA fragmentation in mice

To examine whether JBP485 could inhibit apoptosis in hepatocytes, the effect of JBP485 on DNA fragmentation was investigated. Hepatocyte DNA fragmentation was assayed by agarose gel electrophoresis (Figure 5). The characteristic DNA ladder was found in the Con A group; however, the DNA ladder was significantly attenuated in the JBP485-treated group, which indicated that JBP485 could inhibit hepatocyte apoptosis induced by Con A injection in mice.

RT-PCR analysis of mRNA of apoptosis-related genes bax and bcl-2

To further confirm the effect of JBP485 on inhibition of apoptosis in Con A-treated mice, we subsequently investigated the expression of the apoptosis-related genes bax and bcl-2 by semiquantitative RT-PCR. The total RNA extracted from each group was loaded into 1% agarose gel. Bands at 28S, 18S and 5S could be clearly observed, which confirmed the integrity of the total RNA (Figure 6). In the RT-PCR, 5 μl bax, bcl-2 and β-actin PCR product were separated by electrophoresis and revealed by ethidium bromide staining. The PCR products of bax, bcl-2 and β-actin were 417 bp, 428 bp and 364 bp in length, respectively (Figure 7a). There was no change in the bcl-2 mRNA level 8 h after Con A administration compared with the normal group. However, after treatment with JBP485, the bcl-2 mRNA level increased compared with the Con A group at 8 h after Con A administration ($P < 0.01$) (Figure 7b). The bax mRNA level in the Con A group increased compared with the normal group, but no significant change was found between the Con A and Con A + JBP485 groups (Figure 7b). The ratio of bcl-2/bax mRNA level, which determines

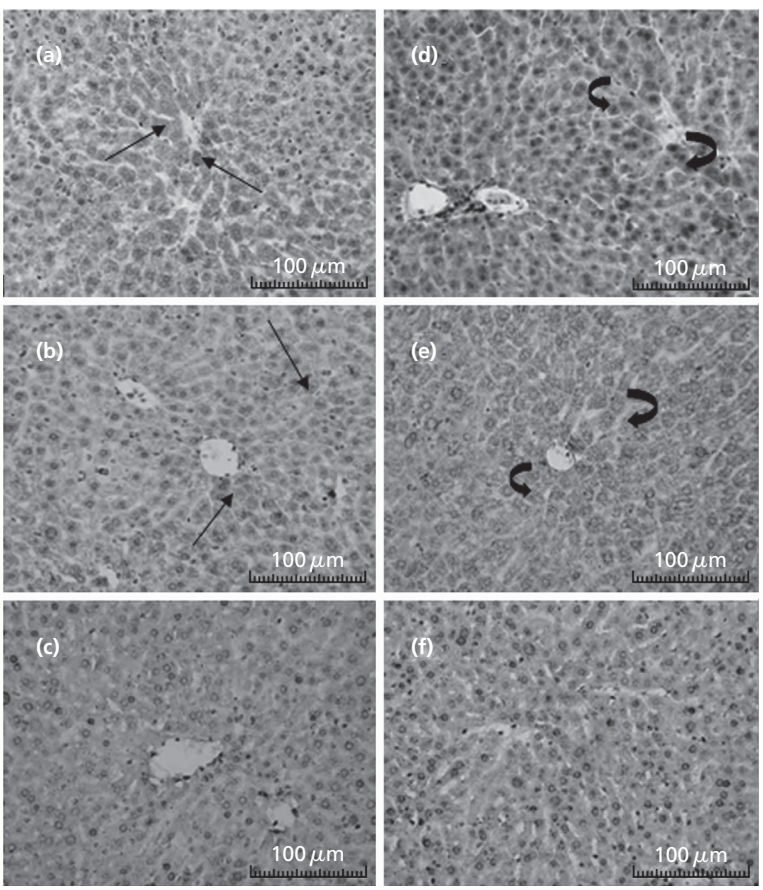

Figure 4 Histopathological changes in liver of mice after induction of liver injury with concanavalin A and treatment with JBP485. Changes in pathology were observed following immunohistochemical staining for tumour necrosis factor-α (TNF-α) (a, b, c) and intracellular adhesion molecule-1 (ICAM-1) (d, e, f) in liver tissue 8 h after concanavalin A (Con A) treatment in BALB/c mice with (b, e) or without (a, d) JBP485 (25 mg/kg). In the Con A group, there was a marked overexpression of TNF-α (straight arrows) and ICAM-1 (curved arrows) in the cytoplasm; these increases were significantly inhibited by JBP485. (c) and (f) represent the normal group. ($n = 6$; magnification $\times$ 400).

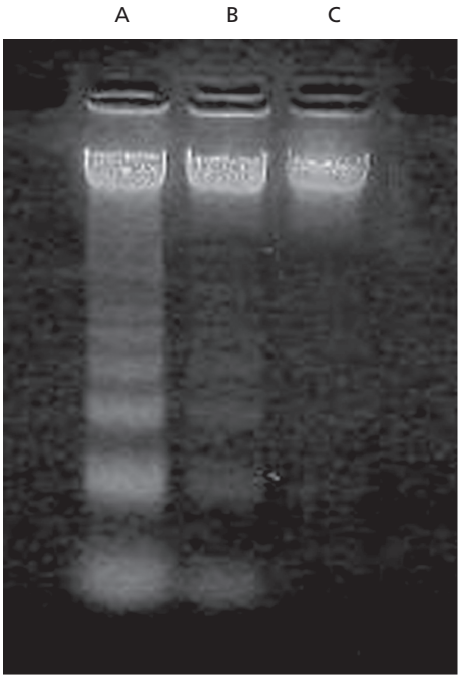

Figure 5 Effect of JBP485 on DNA fragmentation in hepatocytes in concanavalin A-induced hepatic injury in mice. Hepatocyte DNA fragmentation was assayed by agarose gel electrophoresis. A significant decrease in DNA fragmentation was noted in JBP485-treated mice. Lane A, Con A group (10 mg/kg); lane B, Con A + JBP485 group (25 mg/kg); lane C, normal group, $n = 3$ per group.

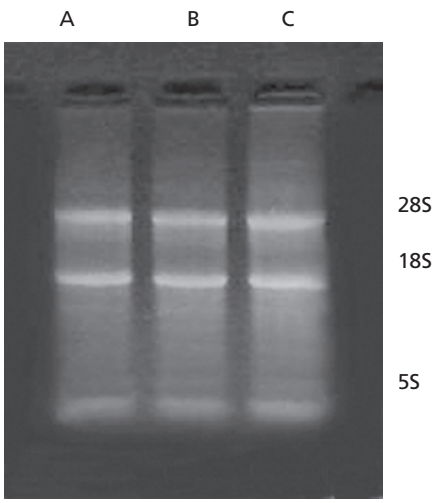

Figure 6 Effect of JBP485 on total RNA in hepatocytes in concanavalin A-induced hepatic injury in mice. Lane A, normal group; lane B, concanavalin A (Con A) alone group; lane C, JBP485 group, $n = 3$ per group.

whether or not apoptosis can occur, was decreased ($P < 0.05$) on exposure to Con A. Compared with the Con A group, the ratio of bcl-2/bax mRNA level in the Con A + JBP484 group was increased ($P < 0.05$) (Figure 7c).

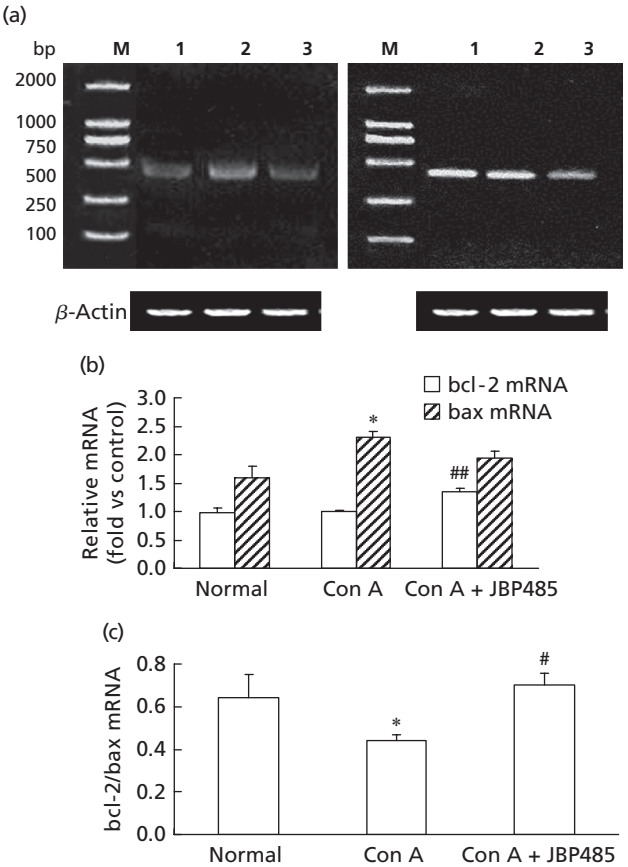

Figure 7 Effect of JBP485 on concanavalin A-induced bcl-2 mRNA and bax mRNA expression in mouse hepatocytes. (a) Reverse transcription-polymerase chain reaction (RT-PCR) analysis of the effect of JBP485 (25 mg/kg) on concanavalin A (Con A)-induced bcl-2 mRNA (left panel) and bax mRNA (right panel) expression in hepatocytes. M, marker; lane 1, Con A group (10 mg/kg); lane 2, Con A + JBP485 group (25 mg/kg); lane 3, normal group. (b) Semiquantitative analysis of RT-PCR products. Densitometric RT-PCR data were standardized with β-actin signals. (c) The ratio of bcl-2 mRNA to bax mRNA. Values are expressed as mean ± SEM, $n = 3$ per group. $^{*}P < 0.05$ vs normal group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs Con A group.

Discussion

Concanavalin A-induced hepatitis in mice and viral hepatitis in humans have several similar pathologic features. These include: inflammatory infiltration; the presence in serum of TNF-α, interferon-γ (IFN-γ) and NO; evidence of apoptotic cell death; augmentation of Fas expression; and the putative involvement of cytotoxic T-cells.[10,11] As such, Con A-induced hepatitis in mice is regarded as an appropriate model for research into the therapy of immune-mediated liver injury, including that associated with viral hepatitis in humans.

In this study, we demonstrated that JBP485 has a protective effect on immune-mediated, Con A-induced hepatitis in mice. Our results indicated that JBP485 significantly inhibited the elevation of serum ALT and LDH levels, which reflects acute disruption of cell membrane integrity. In addition, the morphological study and the determination of MPO suggested

that JBP485 prevented Con A-induced liver injury and inflammatory infiltration in mice.

To further study the protective mechanisms, we then determined the SOD activity and MDA production in liver tissue. We found that JBP485 prevented the decrease in SOD and decreased MDA levels in liver tissue in Con A-intoxicated mice; it suggested that JBP485 exhibited antioxidant activity after oral administration, which partly explained its protective effects in experimentally induced hepatitis.

We also found that JBP485 was able to decrease the levels of nitrite in liver tissue in Con A-intoxicated mice, which indicates that JBP485 could inhibit the over-expression of NO derived from inducible nitric oxide synthase (iNOS) in liver tissue. (One of three different isoforms of NOS is iNOS, which is inducible by cytokines (e.g., TNF-α and IFN-γ) and is able to produce large amounts of NO. As previous studies have reported, liver protection, as well as liver toxicity, has been attributed to NO.)[12] This protective function was observed in alcoholic hepatitis, after partial hepatectomy and after GalN/TNF-α or CCl_4 treatment; the hepatotoxic effect of iNOS expression has been reported in haemorrhagic shock, ischaemia/reperfusion injury, endotoxaemia or after GalN/lipopolysaccharide treatment.[13] Over-expression of iNOS has been seen in many acute and chronic diseases (e.g. septic shock, haemorrhagic shock and hepatitis). Studies have found that excessive NO production derived from iNOS plays an important role in the induction of toxin-induced liver injury.[6,13] NO mediates tissue injury through various pathways, including inhibition of mitochondrial respiration, inactivation of proteinase inhibitors and formation of free radicals.[14] In this study in mice, we demonstrated that JBP485 suppressed the production of nitrite in liver tissue compared with the Con A group, which indicates that the protective effect of JBP485 on Con A-induced hepatitis involves the inhibition of NO production, thereby decreasing the toxicity of hepatocytes in Con A-intoxicated mice.

TNF-α is a central mediator of hepatic inflammation in Con A-induced hepatitis.[15,16] Con A-induced hepatitis has recently been reported to depend critically on Kupffer cell-derived TNF-α.[17] Inhibition of TNF-α by means of antibodies or soluble decoy receptors has proven to have a clinical benefit in patients suffering from immune-mediated diseases, such as inflammatory bowel disease or rheumatoid arthritis.[9] In our models of Con A-induced hepatitis, we tested the therapeutic efficacy of JBP485 in both rat and mouse models of acute inflammatory liver injury induced by Con A.[3] One possible mechanism for the anti-inflammatory activity of JBP485 is that it may inhibit the production and secretion of TNF-α to inhibit the migration of T-lymphocytes, since induction of experimental immune-mediated hepatic damage requires extravasation and migration of activated T-cells; JBP485 could prevent this early event in the evolution of Con A-induced liver damage. An alternative mechanism is indirect inhibition of the production of NO and oxygen-derived free radicals to protect hepatocytes from Con A-induced liver injury.

The active mode of JBP485 in the prevention of Con A-induced liver injury also involves inhibiting ICAM-1 expression. ICAM-1 expression by hepatocytes correlates well with the degree of liver inflammation; hepatocytes express ICAM-1 and vascular cell adhesion molecule-1 after stimulation with lymphokines (e.g. TNF-α) and then T-lymphocytes bind to the hepatocytes via these adhesion molecules. The induction and maintenance of inflammation requires the adhesion of lymphocytes to target cells. Con A induces direct biological change in hepatocytes, resulting in hepatic injury by activated lymphocytes.[18] The pro-inflammatory state is an early step in the inflammatory cascade that promotes the attraction, adherence and subendothelial migration of mononuclear cells to the liver.[16] The expression of the adhesion molecules is a hallmark event in the development of the pro-inflammatory state in endothelial cells. In the Con A-induced liver injury mouse model, we detected over-expression of ICAM-1, which was decreased in the JBP485-treated group. This indicated that JBP485 could suppress lymphocyte adhesion to the endothelium and inhibition of the migration of lymphocytes from blood vessels and penetration of the subendothelium.

Many factors could induce hepatocyte apoptosis, such as oxygen-derived free radicals, NO and TNF-α.[14,15,19] We found that JBP485 markedly inhibited the DNA ladder induced by Con A. To further confirm the exact molecular mechanism of JBP485 inhibition of fragmentation, we subsequently investigated the expression of the apoptosis-related genes bax and bcl-2, which both belong to the bcl-2 gene family. The modulation of the expression of the bcl-2 gene family is an important molecular mechanism for cell apoptosis, which plays a critical role in the common pathway of apoptosis.[19] The biological function of Bcl-2 protein is to protect against many apoptotic factors, counteract the effect of apoptosis and extend lifespan; by contrast, Bax protein, which is highly structurally related to Bcl-2, promotes apoptosis in the Bcl-2 protein family.[19,20] Bax inactivates Bcl-2 by binding to it, to form a heterodimer. Therefore, the ratio of bcl-2 to bax mRNA is a pivotal factor in determining whether or not apoptosis occurs in cells exposed to many injurious agents.[20] Our study suggested that JBP485 is able to protect hepatocytes against apoptosis, mainly by upregulating the expression of the bcl-2 gene to increase the bcl-2/bax mRNA ratio.

Conclusions

Our data demonstrate that JBP485 has a hepatoprotective effect on Con A-induced liver injury in mice. The mechanism can be explained by: (1) inhibition of expression of several inflammatory mediators and cytokines; (2) antioxidant activity related to SOD and MDA; (3) action against apoptosis of hepatocytes by decreasing DNA fragmentation and upregulating the expression of the bcl-2 gene.

Declarations

Conflict of interest

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

Funding

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