Protective Effect of Iridoid Glucosides from *Boschniakia rossica* on Acute Liver Injury Induced by Carbon Tetrachloride in Rats

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Received October 22, 2008; Accepted December 12, 2008; Online Publication, April 7, 2009

[doi:10.1271/bbb.80757]

The protective effect of iridoid glucosides from *Boschniakia rossica* (BRI) against carbon tetrachloride (CCl4)-induced liver injury was examined. CCl4 at a dose of 0.5 ml/kg of body weight was given intraperitoneally to rats to induce liver damage. The rats were sacrificed 16 h after the CCl4 injection. The CCl4 challenge caused a marked increase in the levels of serum amino transferases, tumor necrosis factor-α (TNF-α) and of hepatic inducible nitric oxide synthase (iNOS) protein, depleted reduced glutathione (GSH), and propagated lipid peroxidation. The liver antioxidative defense system, including superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR), as well as the cytochrome P450 2E1 (CYP2E1) expression were suppressed, however. Pre-administration of BRI reversed the significant changes of all liver function parameters induced by CCl4 and restored the liver CYP2E1 content and function. These results demonstrate that BRI produced a protective action on CCl4-induced acute hepatic injury via reduced oxidative stress, suppressed inflammatory response and improved CYP2E1 function in the liver.

Key words: carbon tetrachloride; hepatoprotection; iridoid; *Boschniakia rossica*; rat

Carbon tetrachloride (CCl4) is a potent hepatoxin producing centrilobular hepatic necrosis which causes liver injury. CCl4-induced liver injury depends on a toxic agent that has to be metabolized by the liver NAPDH-cytochrome P450 enzyme system to a highly reactive intermediate.1 It has been suggested that this toxic intermediate is the trichloromethyl radical (CCl3·), producing maximum damage to the liver.2 Antioxidants and anti-inflammatory agents play a critical role in liver protection by scavenging active oxygen and free radicals and neutralizing lipid peroxides.3 Therefore, there is need for a natural product that protects the liver, but is also cost-effective and safe, without side effects. This study evaluated the liver protective potential of iridoid glucosides from *Boschniakia rossica* (BRI), a strong antioxidant and anti-inflammatory medical herb.

*Boschniakia rossica* Fedtsch. et Flerov (Orobanchacea) is a parasitic plant growing on the root of plants of the genus *Alnus* (Betulaceae).5 *Boschniakia rossica* is called an “anti senility herb” because it has effects of tonifying the kidney and strengthening Yang, and is widely used in traditional Chinese medicine as a substitute for *Cistanchis Herba*, a well-known stimatonic agent used in oriental medicine. However, the tonic principle has not yet been well described. We have previously discovered that a crude extract from *Boschniakia rossica* exerted a variety of pharmacological activities, including antioxidative, anti-inflammatory and anti-tumor activities, in animal models.5–7 The chemical constituents of *Boschniakia rossica* were also investigated, which led to the isolation of several phenylpropanoid and iridoid compounds.8–10 Based on the data from our laboratory and others, we have suggested that iridoid compounds from *Boschniakia rossica* may be effective in protecting the liver against acute CCl4 toxicity. So far, however, there has been little research reported on the *in vivo* hepatoprotective effect of iridoid compounds from *Boschniakia rossica*. To test our hypothesis, a classic CCl4-induced liver injury model was chosen to study the liver protective effect of BRI, mainly consisting of boschnaloside and 8-epideoxyloganic acid, in rats.

On the other hand, silymarin, a polyphenolic flavonoid isolated from milk thistle (*Silybum marianum*), is used clinically in Europe and Asia for the treatment of liver diseases. Various studies have indicated that silymarin exhibited strong antioxidative activity,11,12 induced superoxide dismutase (SOD),13 and increased the cellular glutathione (GSH) content.13 By inhibiting lipid peroxidation, silymarin protects against the hepatic toxicity induced by a wide variety of agents.14,15 Pharmacological studies have indicated that silymarin was not toxic, even at high doses.16

To evaluate the hepatoprotective mechanism of BRI, serum tumor necrosis factor-α (TNF-α), hepatic inducible nitric oxide synthase (iNOS), liver oxidative stress and the antioxidative defense system, as well as cytochrome P450 2E1 (CYP2E1), were determined.

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Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BRI, iridoid glucosides from *Boschniakia rossica*; CCL4, carbon tetrachloride; CMC, carboxy methyl cellulose; Cu/Zn-SOD, copper/zinc-superoxide dismutase; CYP2E1, cytochrome P450 2E1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; iNOS, inducible nitric oxide synthase; LOOH, lipid hydroperoxide; MDA, malondialdehyde; Mn-SOD, manganese-superoxide dismutase; PNP, p-nitrophenol; SII, silymarin; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; TNF-α, tumor necrosis factor-α
The effect of BRI on CCl₄-induced acute liver injury was also compared to that of silymarin in this study.

Materials and Methods

Animals. Male Wistar rats weighing 160–180 g were obtained from the animal house section of Yanbian University Health Science Center, China. The animals were distributed in stainless steel metabolic cages and observed under a natural light-dark cycle in a well-ventilated room at 23 ± 1°C. They were fed with standard pellet food and tap water ad libitum. The composition of the control diet was as follows (g/kg): casein, 250; corn starch, 300; sucrose, 250; cellulose powder, 50; corn oil, 100; mineral mixture, 35; vitamin mixture, 10; methionine, 3; choline bitartrate, 2. The composition of the mineral mixture was according to AIN-93G MX and that of the vitamin mixture to AIN-93G VX. The food and water intake was assessed daily for all the animals during the treatment. The experimental procedures were in accordance with internationally accepted guidelines for animal use and care (ECC Directive of 1986; 86/09/EEC; National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, revised 1988) with the approval of the local ethics committee.

Preparation of the test substances. The whole plant of Boschniakia rossica was obtained from Changbai Mountains in China and identified and authenticated by Dr. Zongzhu Yin of Yanbian University, Yanji, Jilin province, China. BRI was extracted by the procedure previously described.10 Briefly, the dried whole plants were extracted with 80% MeOH and evaporated in vacuo to give a crude extract which was successively partitioned with CH₃Cl2 and H₂O. The aqueous layer (16.9% yield) was subjected to column chromatography over MCI-gel CHP20P (Mitsubishi Chemical Co.) with aqueous MeOH in a decreasing polarity (10%, 30%, 50%, 70% and 100% MeOH). The fraction of 50% MeOH (8.6% yield) was collected to give the iridoid glucoside extract, which mostly consisted of iridoid glucosides boschnaloside (49.1%) and 8-epideoxyloganic acid (30.3%) according to the HPLC analysis, and is referred to hereafter as BRI.

Experimental protocol. A total of 50 rats were assigned to five groups as follows: group I, normal control rats with an i.g. 0.5% CMC treatment; group II, the model group with an i.g. 0.5% CMC and CCl₄ treatment; group III, the low-dose BRI group with an i.g. 100 mg/kg BRI and CCl₄ treatment; group IV, the high-dose BRI group with an i.g. 200 mg/kg BRI and CCl₄ treatment; group V, the silymarin group with an i.g. 50 mg/kg silymarin and CCl₄ treatment. All rats were pretreated (administered) with the vehicle, silymarin or BRI suspension daily for a period of 7 d. The silymarin or BRI suspension for intra-gastric administration was prepared by suspending in 0.5% CMC. At 1 h after the last pretreatment, 50% CCl₄ in olive oil was given intraperitoneally to the rats of groups II–V at a dose of 1 ml/kg of body weight, while olive oil (vehicle) was injected to group I.

Blood and liver preparation. Sixteen hours after the administration of CCl₄, the rats were anesthetized with sodium pentobarbital (50 mg/kg of body weight, i.p.) and then sacrificed by cervical decapitation. Blood was collected and allowed to clot at room temperature and then centrifuged to obtain the serum. The whole liver was excised, weighed, and stored at −70°C until needed. The liver was homogenized in 50 mmol/l phosphate-buffered saline (pH 7.2) in an ice bath, and the homogenate was centrifuged at 600 × g for 10 min at 4°C. The supernatant was used for the hepatic biochemical assays and western blot analysis of iNOS.

The supernatant was further centrifuged at 10,000 × g for 20 min to separate the mitochondrial pellet and cytosolic fraction. The cytosolic fraction was used to test the copper/zinc-superoxide dismutase (Cu/Zn-SOD) activity. The mitochondrial pellet was resuspended in a phosphate buffer, and the suspended solution was sonicated for three pulses. The sonicated mitochondria were centrifuged at 8000 × g for 10 min to sediment the unfragmented mitochondria, and the supernatant was used to test the manganese-superoxide dismutase (Mn-SOD) activity.

Liver microsomes for the western blot analysis of CYP2E1 were prepared as described by Funae and Imaoka.19 The liver homogenate was centrifuged at 9000 × g for 20 min, and the resulting supernatant was further centrifuged at 105,000 × g for 60 min. The resulting pellets (microsomes) were resuspended in a 50 mmol/l sodium phosphate buffer (pH 7.4). All procedures were carried out under cold conditions.

Serum marker enzymes and albumin (ALB) assays. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and ALB were determined in accordance with the methods provided by diagnostic kits (Nanjing Jiaocheng Bioengineering Institute, China). Triplicate assays were performed for each measurement, and the average counts were obtained from each individual sample. The inhibitory ratios of BRI in ALT, AST and ALB were calculated by using the following equation:

\[(\text{ALT/AST/ALB of model group} - \text{ALT/AST/ALB of BRI group}) \times 100\%\]

Serum TNF-α assay. The serum concentration of TNF-α was measured by using a rat TNF-α ELISA kit (R&D Systems, USA) according to the corresponding protocol.

Oxidative damage assays. Hepatic and serum lipid hydroperoxide (LOOH) was determined enzymatically, using a kit according to the manufacturer’s instructions (Kyowa Medex Company Ltd., Japan). Malondialdehyde (MDA), a degrading product of lipid peroxidation known as thiobarbituric acid-reactive substances (TBARS), was also determined according to the thiobarbituric acid methods. The separated proteins were electrotransferred to nitrocellulose membranes (Schleicher and Schuell, Germany), using a Trans-Blot Semi-Dry Electrophoretic Transfer Cell (Bio-Rad, USA). The separated proteins were electrotransferred to nitrocellulose membranes (Schleicher and Schuell, Germany), using a Trans-Blot Semi-Dry Electrophoretic Transfer Cell (Bio-Rad, USA). The membranes were blocked with 5% non-fat dry milk for 1 h, and the immunoblots were exposed to the mouse polyclonal antibody against iNOS (Transduction Laboratories, USA) and the rabbit polyclonal antibody against CYP2E1 (Abcam, UK). Immunodetection was performed by using the alkaline phosphatase-labeled goat anti-rabbit IgG antibody and developed with a mixture of 5-bromo-4-chloroindolylphosphate and nitro blue tetrazolium. The filter images were captured by a Gel Doc Image Analysis System (Kodak, Japan). The relative levels of iNOS and CYP2E1 were normalized to glyceralde-
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CYP2E1-specific monooxygenase activity. The CYP2E1-specific monooxygenase activity was measured from the rate of oxidation of p-nitrophenol (PNP) to p-nitrophenate in the presence of NADPH according to the method of Reinke and Moyer. The reaction was performed with 0.1 mg of liver microsomal protein for 30 min at 37 °C, and the absorbance was determined at 510 nm after neutralization. The results are expressed as nmol/min/mg of protein.

Statistical analyses. Data from the experiments are expressed as the mean ± SEM of 10 rats in each group. Statistical analyses were conducted by a one-way analysis of variance followed by Tukey’s post-hoc test by using Statistics Package for Social Science 11.5 software (SPSS Inc., USA). *P* < 0.05 is considered statistically significant.

Results

The body weight of the rats continued to increase in all groups, but no difference was apparent among the groups throughout the experimental period. Neither the food intake nor water intake was significantly different among the groups during the experiment, and no mortality occurred throughout the study (data are not shown).

Table 1 shows that the rats of the model group (group II) induced with a single dose of CCl₄ developed hepatic damage as compared with the normal control group (group I). This was evident from marked modifications of liver function parameters AST, ALT and ALB in the serum. BRI and silymarin administration showed a significant reduction in AST and ALT activities with an elevated ALB level as compared with the CCl₄-intoxicated model group. In the rats of groups III and IV, the oral administration of BRI at doses of 100 and 200 mg/kg prior to the CCl₄ challenge was observed to dose-dependently reverse the CCl₄-induced alteration of ALT by 49% and 70%, of AST by 28% and 37%, and of ALB by 34% and 85%, respectively.

The CCl₄ treatment also induced an increase in the serum TNF-α level as compared with the normal control group. However, the serum TNF-α level in both the 200 mg/kg BRI and silymarin-supplemented groups was significantly lower than that in the CCl₄-treated group (Table 1).

An expected increase of the hepatic and serum lipid peroxidative indices in the CCl₄-treated model group also confirmed that oxidative damage had been induced (Fig. 1). When CCl₄ was injected into the rats that had been pretreated with 200 mg/kg of BRI or silymarin, the levels of LOOH and MDA in the liver and serum were significantly lower than those in the CCl₄-treated model group. The observed suppression of oxidative damage in the CCl₄-injured liver and serum by BRI preadministration suggests that BRI was antioxidative and hepatoprotective.

Figure 2 shows that the hepatic and serum GSH levels were markedly lower in the CCl₄-intoxicated rats. Supplementation with 200 mg/kg of BRI restored the decreased level of GSH caused by the CCl₄ injection to the normal level. The GSH levels between the BRI-supplemented groups were also significantly different.

Beyond the decreased GSH level, CCl₄ also induced substantial modifications to the hepatic antioxidative enzymes (Table 2). The data show that the decreased levels of hepatic SOD, Mn-SOD and GR activity as the result of CCl₄ injection were significantly elevated in the BRI and silymarin groups. The CAT, GPX and Cu/Zn-SOD activities also tended to decrease to a small extent in the CCl₄-treated model control group, although the differences were not statistically significant as compared with the normal control group. Interestingly, the CAT and GPX activities of the 200 mg/kg BRI group were markedly higher than those of the CCl₄-treated group, being at even higher levels than those of the

**Table 1.** Effect of BRI against CCl₄-Induced Alteration of the Liver Function Parameters in Serum

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALB (g/dl)</th>
<th>TNF-α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>42.3 ± 12.4</td>
<td>101.2 ± 38.6</td>
<td>4.48 ± 1.04</td>
<td>42.2 ± 18.4</td>
</tr>
<tr>
<td>II</td>
<td>120.9 ± 24.7</td>
<td>244.8 ± 36.7</td>
<td>3.35 ± 0.75</td>
<td>112.7 ± 31.2</td>
</tr>
<tr>
<td>III</td>
<td>82.4 ± 21.3</td>
<td>205.1 ± 56.9</td>
<td>3.74 ± 0.77</td>
<td>89.3 ± 24.6</td>
</tr>
<tr>
<td>IV</td>
<td>63.1 ± 22.6</td>
<td>191.4 ± 53.7</td>
<td>4.32 ± 0.74</td>
<td>70.1 ± 23.3</td>
</tr>
<tr>
<td>V</td>
<td>71.8 ± 20.2</td>
<td>179.1 ± 48.6</td>
<td>4.05 ± 0.71</td>
<td>69.7 ± 24.0</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SEM (n = 10) in each group. The animals were pretreated with BRI or silymarin or a vehicle. CCl₄ was given i.p. on the last day of the treatment protocol, and the animals were sacrificed 16 h after CCl₄ administration. *P* < 0.05, significantly different from group II. Group I, normal control; group II, model (CCl₄ alone); group III, CCl₄ + 100 mg/kg of BRI; group IV, CCl₄ + 200 mg/kg of BRI; group V, CCl₄ + 50 mg/kg of silymarin.
nor-normal control group. However, no dose-dependent differences were apparent between groups III and IV in the levels of hepatic antioxidant enzyme activities.

CCl₄-induced hepatotoxicity is also accompanied by the production of proinflammatory mediators such as iNOS. The western blot analysis revealed that the amount of hepatic iNOS protein increased markedly after the CCl₄ administration. *P < 0.05, significantly different from group II; group I, normal control; group II, model (CCl₄ alone); group III, CCl₄ + 100 mg/kg of BRI; group IV, CCl₄ + 200 mg/kg of BRI; group V, CCl₄ + 50 mg/kg of silymarin.

Discussion

Pharmacological studies on iridoid glucosides from medicinal plants have revealed that they exhibit antioxidative, anti-inflammatory and anti-hepatotoxic activities. 15,16 Various plants used in traditional medicine contain significant amounts of iridoid glucosides. 8–10,21,22 Some iridoid and phenylpropanoid compounds have recently been isolated from Boschniakia rossica and presumed to be responsible for its major pharmacological functions. 5–7,21 The present in vivo study has demonstrated the hepatoprotective potential of the iridoid compounds from Boschniakia rossica.

The liver injuries induced by CCl₄ are the best characterized system for xenobiotic-induced hepatotoxicity and are commonly used models for screening of the anti-hepatotoxic and/or hepatoprotective activities of drugs. 23 Cellular injury occurs when free radical generation exceeds the cellular capacity of its removal. Cells have a number of mechanisms to protect themselves from the toxic effects of free radicals, including free radical scavengers and chain reaction terminators such as SOD, CAT, GPx and GR. 24,25 GSH in the liver plays a primary role in the protection against trichloromethyl radical-induced liver damage. 2,25 It has been suggested that the lipid peroxides generated after CCl₄ intoxication are eliminated by GPx in the presence of GSH, thus curbing the propagation of lipid peroxidation. 25 In this study, hepatocellular damage induced by CCl₄ intoxication in rats was established from significant alterations in the serum ALT, AST and ALB levels.

Table 2. Effect of BRI against CCl₄-Induced Alterations of the Hepatic Antioxidative Enzyme Activities

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD</th>
<th>Cyt/Zn-SOD</th>
<th>Mn-SOD</th>
<th>GPx</th>
<th>GR</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10.2 ± 2.2</td>
<td>1.71 ± 0.38</td>
<td>11.8 ± 1.7</td>
<td>7.7 ± 1.1</td>
<td>6.5 ± 0.4</td>
<td>8.2 ± 1.4</td>
</tr>
<tr>
<td>II</td>
<td>3.9 ± 0.9</td>
<td>1.50 ± 0.37</td>
<td>4.5 ± 1.7</td>
<td>6.7 ± 0.6</td>
<td>4.3 ± 0.8</td>
<td>7.6 ± 1.5</td>
</tr>
<tr>
<td>III</td>
<td>5.4 ± 1.2</td>
<td>1.58 ± 0.43</td>
<td>6.8 ± 1.1</td>
<td>7.4 ± 0.9</td>
<td>4.8 ± 0.7</td>
<td>10.4 ± 1.1*</td>
</tr>
<tr>
<td>IV</td>
<td>7.1 ± 1.5*</td>
<td>1.55 ± 0.26</td>
<td>7.9 ± 1.8*</td>
<td>9.1 ± 1.5*</td>
<td>5.3 ± 0.9*</td>
<td>11.4 ± 1.2*</td>
</tr>
<tr>
<td>V</td>
<td>9.7 ± 2.1*</td>
<td>1.59 ± 0.42</td>
<td>10.6 ± 2.0*</td>
<td>7.2 ± 0.7*</td>
<td>5.6 ± 1.1*</td>
<td>12.5 ± 1.7*</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SEM (n = 10) in each group. Values are expressed as U/mg of protein for SOD, CAT, GPx and GR. The animals were pretreated with BRI, silymarin or a vehicle. CCl₄ was given i.p. on the last day of the treatment protocol, and the animals were sacrificed 16 h after CCl₄ administration. *P < 0.05, significantly different from group II; Group I, normal control; group II, model (CCl₄ alone); group III, CCl₄ + 100 mg/kg of BRI; group IV, CCl₄ + 200 mg/kg of BRI; group V, CCl₄ + 50 mg/kg of silymarin.

Fig. 2. Effect of BRI on the CCl₄-Induced Hepatic and Serum GSH Levels in Rats.

Data are presented as the mean ± SEM (n = 10) in each group. The animals were pretreated with BRI, silymarin or a vehicle. CCl₄ was given i.p. on the last day of the treatment protocol, and the animals were sacrificed 16 h after CCl₄ administration. *P < 0.05, significantly different from group II; ?P < 0.05, significantly different from group III. Group I, normal control; group II, model (CCl₄ alone); group III, CCl₄ + 100 mg/kg of BRI; group IV, CCl₄ + 200 mg/kg of BRI; group V, CCl₄ + 50 mg/kg of silymarin.

Fig. 3. Effect of BRI on the Hepatic iNOS Protein Expression.

Data are presented as the mean ± SEM (n = 6) in each group. The animals were pretreated with BRI, silymarin or a vehicle. CCl₄ was given i.p. on the last day of the treatment protocol, and the animals were sacrificed 16 h after CCl₄ administration. *P < 0.05, significantly different from group II. Group I, normal control; group II, model (CCl₄ alone); group IV, CCl₄ + 200 mg/kg of BRI; group V, CCl₄ + 50 mg/kg of silymarin.

SIL, silymarin.
as found by previous researchers.\textsuperscript{17,26} However, the serum ALT and AST activities significantly declined in the BRI-supplemented groups, while the serum ALB level elevated, suggesting that BRI was beneficial for liver regeneration to reverse liver injury. In parallel with the alteration of liver function markers, increased production of LOOH and MDA, GSH depletion, and decreased activities of SOD, Mn-SOD and GR occurred in the liver. Our results show that the BRI-pretreated animals demonstrated a significant reduction in the levels of hepatic and serum peroxidative markers with a concomitant improvement in the activities of the hepatic antioxidative defense system. This suggests that BRI contained some bioactive components, namely boschnaloside and 8-epideoxyloganic acid, that may have been able to protect against the oxidation of hepatic cellular membrane damage via a free radical scavenging property.

The results also show that BRI suppressed an increase in the serum TNF-\(\alpha\) level. TNF-\(\alpha\) is a proinflammatory cytokine produced predominantly by macrophages and plays a key role in the host defense response to injury and infection. However, excessive, prolonged production of TNF-\(\alpha\) is thought to contribute to the pathology of liver damage and systematic toxicity.\textsuperscript{27} Following the administration of CCl\(_4\), the hepatic expression of TNF-\(\alpha\) increased abnormally in this study. The BRI pretreatment inhibited the elevated TNF-\(\alpha\) production following CCl\(_4\) injection, and the low level of TNF-\(\alpha\) might have played a protective role in this model by inducing hepatocyte proliferation and the release of mediators involved in tissue repair. It is also possible that the increased serum TNF-\(\alpha\) level induced by CCl\(_4\) was significantly reduced by BRI supplementation due to its anti-inflammatory effect, and thus inhibited the production of TNF-\(\alpha\) from Kupffer cells. Further studies may be needed to elucidate this phenomenon.

**Fig. 4.** Effect of BRI on the Hepatic CYP2E1 Protein Expression.

Data are presented as the mean \(\pm\) SEM (\(n = 6\)) in each group. The animals were pretreated with BRI, silymarin or a vehicle. CCl\(_4\) was given i.p. on the last day of the treatment protocol, and the animals were sacrificed 16 h after CCl\(_4\) administration. *\(P < 0.05\), significantly different from group II. Group I, normal control; group II, model (CCl\(_4\) alone); group IV, CCl\(_4\) + 200 mg/kg of BRI; group V, CCl\(_4\) + 50 mg/kg of silymarin. SIL, silymarin.

TNF-\(\alpha\) also stimulates the release of cytokines from macrophages and induces phagocyte oxidative metabolism and nitric oxide production.\textsuperscript{28} Nitric oxide can exacerbate oxidative stress by reacting with reactive oxygen species, particularly with the superoxide anion, and forming peroxynitrite.\textsuperscript{29} However, as nitric oxide has a range of effects on a variety of physiological processes,\textsuperscript{30} it is unclear if it is beneficial or detrimental to the liver injury induced by hepatotoxins. This study has confirmed a significant increase in iNOS protein expression in the liver after CCl\(_4\) administration. These alterations were attenuated by the 200 mg/kg BRI treatment which suggests that BRI suppressed iNOS protein secretion and/or enhanced the degradation of their proteins.

It has been established that CCl\(_4\) hepatotoxicity depends on its reductive dehalogenation catalyzed by the cytochrome P450 (CYP) system in the endoplasmic reticulum of hepatic cells, leading to the generation of an unstable complex trichloromethyl radical.\textsuperscript{1,2} The trichloromethyl radical may bind either at the heme group of CYP or at the active site of the enzyme near the heme group, leading to inactivation of the CYP pathways.\textsuperscript{25} In the present study, a significant decrease in the hepatic CYP2E1 protein content and CYP2E1-specific PNP hydroxylase activity was found in the rats treated with CCl\(_4\) alone. In contrast, in the rats that received BRI, the hepatic CYP2E1 protein content and PNP hydroxylase activity were less suppressed after the CCl\(_4\) treatment. This result suggests that BRI exerted a beneficial effect on hepatic CYP2E1 restoration. It is possible that BRI may restore the hepatic CYP2E1 function and lead to a rapid recovery from CCl\(_4\)-induced liver injury. Similar observations regarding a decrease in the CYP2E1 protein content have been found in rats treated with \(\delta\)-galactosamine\textsuperscript{31} or CCl\(_4\).\textsuperscript{32} In these studies, an increase in the CYP2E1 protein content following pharmacological manipulation ameliorated the hepatotoxicity after a \(\delta\)-galactosamine or CCl\(_4\) administration. However, it is also possible that the administration of BRI caused a reduction of CCl\(_4\)-
related hepatotoxicity that led to early recovery of the CYP2E1 protein content in the rats receiving BRI. Therefore, decreased hepatotoxicity following BRI administration would lead to an elevation of the liver enzyme activity.

In conclusion, BRI had a protective effect on CCl\textsubscript{4}-induced acute liver injury, at least in part, by reducing oxidative stress, suppressing the inflammatory response and restoring the CYP2E1 function.

Acknowledgments

This study was supported by a grant from the National Natural Science Foundation of China (no. 30360113).

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