Attenuation by Oren-gedoku-to Extract (TJ-15) of Disruption of Hepatic Reactive Oxygen Species Metabolism with Progression of Carbon Tetrachloride-Induced Acute Liver Injury in Rats

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Summary We attempted to elucidate how Oren-gedoku-to (Huanglian-Jie-Du-Tang) extract (TJ-15), a Chinese herbal medicine, attenuates the disruption of hepatic reactive oxygen species metabolism with the progression of carbon tetrachloride (CCl4)-induced acute liver injury in rats. TJ-15 (100, 250 or 500 mg/kg body weight) was orally administered to rats injected with CCl4 (1 ml/kg, i.p.) at 6 h after the toxicant treatment. Post-administered TJ-15 reduced progressive liver injury in CCl4-treated rats at 24 h after the toxicant treatment dose-dependently. The liver of rats treated with CCl4 alone showed increases in the concentration of thiobarbituric acid-reactive substances, an index of lipid peroxidation, and xanthine oxidase activity and decreases in reduced glutathione and ascorbic acid concentrations and superoxide dismutase, catalase, and glutathione reductase activities at 24 h after the toxicant treatment. The liver of CCl4-treated rats showed no change in Se-glutathione peroxidase activity and an increase in hepatic glucose-6-phosphate dehydrogenase activity at 24 h after the toxicant treatment. Post-administered TJ-15 attenuated the increases in hepatic thiobarbituric acid-reactive substances and xanthine oxidase activity and the decreases in hepatic reduced glutathione and ascorbic acid concentrations and superoxide dismutase, catalase, and glutathione reductase activities dose-dependently but did not affect the hepatic Se-glutathione peroxidase activity and the increased hepatic glucose-6-phosphate dehydrogenase activity. These results indicate that orally administered TJ-15 attenuates the disruption of hepatic reactive oxygen species metabolism with the progression of CCl4-induced acute liver injury in rats through its direct and indirect antioxidant actions. The results also suggest that this attenuating effect of TJ-15 could contribute to its preventive effect on the progression of CCl4-induced acute liver injury.

Key Words: Oren-gedoku-to (Huanglian-Jie-Du-Tang) extract (TJ-15), carbon tetrachloride, liver injury (rat), reactive oxygen species metabolism

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Introduction

Oren-gedoku-to (Huanglian-Jie-Du-Tang), a traditional Chinese herbal medicine, is the boiled extract of four herbs, i.e., *Coptidis Rhizoma*, *Scutellariae Radix*, *Phellodendri Cortex*, and *Gardeniae Fructus*, and has been used for the therapies of hepatitis and liver dysfunction in addition to gastric ulcers, dermatitis, dementia, and cerebrovascular diseases in Japan [1]. Oren-gedoku-to extract (Tsumura TJ-15) exerts an antioxidant action by scavenging reactive oxygen species (ROS) such as superoxide radical (O$_2^-$) and hydroxyl radical (‘OH) [2–4] and by inhibiting lipid peroxidation in vitro [5, 6].

It has been shown that hepatic lipid peroxidation increases at a progressed stage of liver injury as well as at an early stage of the injury in rats treated once with carbon tetrachloride (CCl$_4$) [7–14]. It has also been shown in rats with a single CCl$_4$ treatment that hepatic antioxidant defense systems associated with antioxidants such as reduced glutathione (GSH) and ascorbic acid and antioxidant enzymes such as superoxide dismutase (SOD), an enzyme to scavenge O$_2^-$, catalase (CAT), an enzyme to decompose hydrogen peroxide (H$_2$O$_2$), and glutathione reductase (GSSG-R), an enzyme to regenerate GSH from oxidized glutathione (GSSG) using NADPH, are disrupted at a progressed stage of liver injury [8, 9, 12–16], although the activity of glucose-6-phosphate dehydrogenase (G-6-PDH), an enzyme to generate NADPH, are disrupted at a progressed stage of liver injury [9, 14, 15, 17]. In addition, our previous report has shown that O$_2^-$ and H$_2$O$_2$ derived from xanthine oxidase (XO) contribute to the progression of CCl$_4$-induced acute liver injury in rats by stimulating lipid peroxidation and by disrupting antioxidant defense systems in the liver tissue [13]. We have reported that a single oral administration of TJ-15 (500 mg/kg body weight) to rats treated once with CCl$_4$ after the appearance of liver injury prevents progressive liver injury with attenuation of decreased hepatic GSH level and SOD activity and increased hepatic lipid peroxide level [9]. Thus, there is a possibility that orally administered TJ-15 attenuates the disruption of hepatic ROS metabolism with the progression of CCl$_4$-induced acute liver injury in rats.

The purpose of the present study was to elucidate how TJ-15 attenuates the disruption of hepatic ROS metabolism with the progression of CCl$_4$-induced acute liver injury in rats. Namely, we examined the attenuating effect of TJ-15 administered orally at a dose of 100, 250 or 500 mg/kg body weight on the hepatic changes in the concentrations of thiobarbituric acid-reactive substances (TBARS), an index of lipid peroxidation, GSH, and ascorbic acid and the activities of SOD, CAT, Se-glutathione peroxidase (Se-GPX), an enzyme to metabolize H$_2$O$_2$ and lipid hydroperoxides using GSH, GSSG-R, G-6-PDH, and XO with the progression of CCl$_4$-induced acute liver injury in rats.

Materials and methods

Materials

TJ-15 was kindly provided by Tsumura & Co. (Tokyo, Japan). TJ-15 is a spray-dried material from Oren-gedoku-to extract. This medicine was prepared as follows: after drying, the four herbs (total amount, 8.5 g), i.e. 2.0 g of *Coptidis japonica* Makino (rhizome), 1.5 g of *Phellodendron amurense* Ruprecht (root), 3.0 g of *Scutellaria baicalensis* Georgi (root), and 2.0 g of *Gardenia jasminoides* Ellis (fruit), were boiled in 10 times the weight of water for 1 h. The resultant extract was spray-dried. The percentage yield was 17.6%. Main components present in the TJ-15 preparation were confirmed by analysis using high-performance liquid chromatography (HPLC) with spectrophotometric detection as follows: TJ-15 preparation (1.0 g) was extracted with methanol (20 ml) under ultrasonication for 30 min. The solution was filtered and then submitted for HPLC analysis. HPLC equipment was controlled with HPLC pump (LC-10AD, Shimazu, Kyoto, Japan) using a TSK-GEL 80TS column (4.6Ø250 cm) (TOSOH, Tokyo, Japan), eluting with solvents (A) 0.05 mM acetic acid-ammonium acetate buffer (pH 3.6) and (B) acetonitrile. A linear gradient of 90% A and 10% B changing over 60 min to 0% A and 100% B was used. The flow rate was 1.0 ml/min. The eluate from the column was monitored in the wavelength range between 200 and 400 nm, and the three-dimensional data were processed by a diode array detector, SPD-M10A (Shimadzu, Kyoto, Japan).

The three-dimensional HPLC chart of the methanol solution of TJ-15 preparation is shown in Fig. 1. This TJ-15 preparation contained berberine (derived from *Coptidis Rhizoma* and *Phellodendri cortex*), coptisine, columbamine, epiberberine, groenlandicine, jateorrhizine, magnoflorine, palmitine (derived from *Coptidis Rhizoma*), baicalein, bicalin, oroxylin A, oroxylin A 7-O-glucuronide, skullcapflavone, wogonin, wogonin 7-O-glucuronide (derived from *Scutellariae Radix*), palmatine (derived from *Phellodendri Cortex*), and geniposide (derived from *Gardeniae Fructus*). Bovine erythrocyte Cu,Zn-SOD, bovine serum albumin, G-6-P, leupeptin, and xanthine were purchased from Sigma (St Louis, MO); phenylmethylsulfonylfluoride, milk XO, and yeast GSSG-R from Roche-Diagnostic (Tokyo, Japan); ascorbic acid, CCl$_4$, 2,2'-dipyridyl, 5,5'-dithiobis(2-nitrobenzoic acid) (Ellman’s reagent), dithiothreitol, ethylenediaminetetraacetic acid (EDTA), GSH, GSSG, NADP$^+$, NADPH, 2-thiobarbituric acid, and other chemicals from Wako (Osaka, Japan).
CCl₄ and TJ-15 treatments

Seven-week-old male Wistar rats weighing 220–230 g, purchased from Nippon SLC (Hamamatsu, Japan), were used. The animals were allowed free access to rat chow (Oriental FM, Oriental Yeast, Tokyo, Japan) and water before CCl₄ treatment but then were starved with free access to water after CCl₄ treatment. Rats received a single i.p. injection of CCl₄ at a dose of 1 ml/kg body weight as a 50% olive oil solution between 9:00 and 10:00 am, as described previously [9, 11, 13, 14]. The CCl₄-untreated rats received an equal volume of olive oil in the same manner. TJ-15 (10, 25 or 50 mg) was suspended in 1.0 ml of distilled water. Rats with and without CCl₄ injection received a single oral administration of TJ-15 (100, 250 or 500 mg/kg body weight) at a dose of 10 ml of each TJ-15 suspension per kg body weight at 6 h after the toxicant injection. TJ-15-untreated rats with and without CCl₄ injection received an equal volume of distilled water at the same time point in the same manner. Rats were sacrificed 6 and 24 h after CCl₄ injection under ether anesthesia, at which time blood was collected from the inferior vena cava. The collected blood was separated into serum by centrifugation. Immediately after sacrifice, livers were perfused with ice-cold 0.15 M KCl and then isolated. The livers and serum were stored at −80ºC until use. All animals received humane care in compliance with the Guideline for the Management of Laboratory Animals in Fujita Health University.

Assays of serum and hepatic components and enzymes

Serum alanine aminotransferase (ALT) and aspartic acid aminotransferase (AST) were assayed using a commercial kit, Iatrozyme TA-LQ (DaiIatron Co., Tokyo, Japan). These enzyme activities are expressed as an international unit (IU/l).

Livers were homogenized in 9 volumes of ice-cold 0.15 M KCl containing 1.0 mM EDTA using a glass homogenizer with a Teflon pestle. This homogenate was used for hepatic TBARS, GSH, and ascorbic acid assays. Hepatic TBARS was assayed by the method of Ohkawa et al. [18] using the thiobarbituric acid reaction except that 1.0 mM EDTA was added to the reaction medium. The concentration of hepatic TBARS is expressed as that of malondialdehyde (MDA) equivalents. Hepatic GSH was assayed by the method of Sedlak and Lindsay [19] using Ellman’s reagent. Hepatic ascorbic acid was assayed by the method of Zannoi et al. [20] using 2,2′-dipyridyl. The prepared liver homogenate was sonicated 2 times on ice for 30 s using a Handy Sonic model UR-20P (Tomy Seiko, Tokyo, Japan). The sonicated homogenate was centrifuged at 12,000 × g for 20 min and the resultant supernatant was dialyzed against 100 volumes of 0.05 M Tris-HCl buffer (pH 7.4) for 1 h using a microdialysis device (molecular weight cut-off = 3,500) (Bio-Tec International, Bellevue, WA). These treatments were performed at 4ºC. The dialyzed supernatant was used for hepatic SOD, CAT, Se-GPX, GSSG-R, and G-6-PDH assays. Hepatic SOD, CAT, Se-GPX, GSSG-R, and G-6-PDH were assayed by the methods of Oyanagui [21], Bergmeyer [22], Hochstein and Utley [23], Lopez-Barea...
Histological examination

Liver samples were taken from the central part of the right large lobe of untreated control rats and CCl₄-treated rats post-administered with and without TJ-15 at 24 h after the intoxication. They were fixed with 10% formalin in phosphate buffered saline for 24 h and then washed with tap water, dehydrated in alcohols, and embedded in paraffin. Sections 6–7 µm thick were mounted in glass slides. Staining with hematoxylin and eosin (H-E) was performed in each slide and then histological examination was conducted under light microscopy.

Statistical analysis

All values obtained are expressed as the mean ± S.D. All data were statistically analyzed by ANOVA (StatView). Each mean value is compared by one-way analysis of variance and Fisher’s protected significant difference for multicomparison as the post hoc test. The level of significance was set at p<0.05.

Results

The CCl₄-treated group had significantly higher serum AST and ALT activities than the control group at 6 and 24 h after the toxicant treatment and the increased serum AST and ALT activities in the CCl₄-treated group were markedly higher at 24 h than at 6 h (Table 1). When TJ-15 at a dose of 100, 250 or 500 mg/kg body weight was orally administered to CCl₄-treated rats at 6 h after the toxicant treatment, the increased serum AST and ALT activities found at 24 h were significantly attenuated in a dosedependent manner (Table 1). Oral administration of TJ-15 to CCl₄-untreated rats at the same doses had no effect on the serum AST and ALT activities (Table 1).

The liver sections stained by H-E in CCl₄-treated rats post-administered with and without TJ-15 (500 mg/kg body weight) and untreated control rats were examined for histological changes at 24 h after CCl₄ intoxication. Hepatocytes in the untreated control group showed little histological changes (Fig. 2A). Hepatocytes in the centrlobular area of the CCl₄-treated group presented necrotic and degenerative changes with severe inflammatory cell infiltration (Fig. 2B). In contrast, hepatocytes in the centrlobular area of the CCl₄-treated group post-administered with TJ-15 presented much less necrotic and degenerative changes and a slight inflammatory cell infiltration (Fig. 2C).

Table 1. Effect of post-administered TJ-15 on serum changes in AST and ALT activities in CCl₄-treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>AST (IU/l)</th>
<th>ALT (IU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 h after CCl₄ treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>61 ± 8</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>CCl₄</td>
<td>5</td>
<td>95 ± 12*</td>
<td>34 ± 5*</td>
</tr>
<tr>
<td>24 h after CCl₄ treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>72 ± 5</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>TJ-15 (100 mg/kg)</td>
<td>5</td>
<td>63 ± 9</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>TJ-15 (250 mg/kg)</td>
<td>5</td>
<td>77 ± 8</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>TJ-15 (500 mg/kg)</td>
<td>5</td>
<td>72 ± 7</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>CCl₄</td>
<td>14</td>
<td>867 ± 195*</td>
<td>421 ± 55*</td>
</tr>
<tr>
<td>CCl₄ + TJ-15 (100 mg/kg)</td>
<td>7</td>
<td>538 ± 94**</td>
<td>261 ± 38**</td>
</tr>
<tr>
<td>CCl₄ + TJ-15 (250 mg/kg)</td>
<td>7</td>
<td>273 ± 93**</td>
<td>95 ± 23**</td>
</tr>
<tr>
<td>CCl₄ + TJ-15 (500 mg/kg)</td>
<td>7</td>
<td>208 ± 92**</td>
<td>71 ± 15**</td>
</tr>
</tbody>
</table>

Rats were intraperitoneally injected with either CCl₄ (1 mg/kg body weight) or vehicle (olive oil) and then received orally either TJ-15 (100, 250 or 500 mg/kg body weight) or vehicle (distilled water) at 6 h after the toxicant treatment. The animals were sacrificed 6 or 24 h after CCl₄ treatment. Each value is a mean ± S.D. with the indicated number (N) of rats. *p<0.05 (vs. control group); **p<0.05 (vs. CCl₄-treated group).

When hepatic XO activity and TBARS, GSH, and ascorbic acid concentrations in rats treated with and without CCl₄ were determined 6 and 24 h after the toxicant treatment, the results shown in Table 2 were obtained. There was no difference in hepatic XO activity between the CCl₄-treated and control groups at 6 h after the toxicant treatment but that activity was significantly higher in the CCl₄-treated group than in the control group at 24 h. At 6 h after CCl₄ treatment, hepatic TBARS concentration was significantly higher in the CCl₄-treated group than in the control group, while hepatic GSH and ascorbic acid concentrations were significantly lower in the treated group than in the control group. The CCl₄-treated group showed further increase in hepatic TBARS concentration and further decreases in hepatic GSH and ascorbic acid concentrations at 24 h after the toxicant treatment. Oral administration of TJ-15 at a dose of 100, 250 or 500 mg/kg body weight to CCl₄-treated rats at 6 h after the toxicant treatment significantly attenuated the increases in hepatic XO activity and TBARS concentration and the decreases in hepatic GSH and ascorbic acid concentrations found at 24 h in a dose-dependent manner (Table 2). The hepatic XO activity and TBARS and GSH concentrations in the CCl₄-treated group post-administered with TJ-15 (500 mg/kg body weight) were not significantly different from those in the control group (Table 2). Oral administration of TJ-15 to CCl₄-untreated rats at the same doses did not affect the hepatic XO activity and TBARS, GSH, and ascorbic acid concentrations (Table 2).

When hepatic SOD, CAT, Se-GPX, GSSG-R, and G-6-PDH activities in rats treated with and without CCl₄ were determined 6 and 24 h after the toxicant treatment, the results shown in Table 3 were obtained. The CCl₄-treated group had significantly lower hepatic SOD activity than the control group at 6 h after the toxicant treatment and this reduction in the CCl₄-treated group was enhanced at 24 h. In the CCl₄-treated group, hepatic CAT and GSSG-R activities did not change 6 h after the toxicant treatment but both enzyme activities decreased significantly at 24 h. Hepatic Se-GPX activity in the CCl₄-treated group did not change 6 and 24 h after the toxicant treatment. Hepatic G-6-PDH activity was significantly higher in the CCl₄-treated group than in the control group at 6 and 24 h after the toxicant treatment and the extent of increase in that activity was similar at 6 and 24 h. Oral administration of TJ-15 (100, 250 or 500 mg/kg body weight) to CCl₄-treated rats at 6 h after the toxicant treatment significantly attenuated the decreases in hepatic SOD, CAT, and GSSG-R activities found at 24 h in a dose-dependent manner (Table 3). The hepatic SOD, CAT, and GSSG-R activities in the CCl₄-treated group post-administered with TJ-15 (500 mg/kg body weight) were not significantly different from those in the control group (Table 3). However, no dose of post-administered TJ-15 had any effect on the hepatic Se-GPX activity and the increased hepatic G-6-PDH activity at 24 h after CCl₄ treatment (Table 3). The same doses of TJ-15 given to CCl₄-untreated rats did not affect the hepatic SOD, CAT, Se-GPX, GSSG-R, and G-6-PDH activities (Table 3).

**Discussion**

In the present study, oral administration of TJ-15 to rats treated once with CCl₄ (1 ml/kg body weight, i.p.) at a dose of 100, 250 or 500 mg/kg body weight after the appearance of liver injury, i.e., at 6 h after the toxicant treatment,
was found to prevent the progression of CCl
dhistological observation. Thus, orally administered TJ-15
progression of CCl\textsubscript{4}
reduced progressive liver injury at 24 h in a dose-dependent
treatment group).

Table 2. Effect of post-administered TJ-15 on hepatic changes in XO activity and TBARS, GSH, and ascorbic acid concentrations in CCl\textsubscript{4}-treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>XO (mU/mg protein)</th>
<th>TBARS (pmol MDA/mg protein)</th>
<th>GSH (nmol/mg protein)</th>
<th>Ascorbic acid (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6 h after CCl\textsubscript{4} treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>0.275 ± 0.038</td>
<td>322 ± 23</td>
<td>49.4 ± 1.4</td>
<td>2.21 ± 0.08</td>
</tr>
<tr>
<td>CCl\textsubscript{4}</td>
<td>5</td>
<td>0.314 ± 0.039</td>
<td>381 ± 45*</td>
<td>38.4 ± 1.2*</td>
<td>1.71 ± 0.05</td>
</tr>
<tr>
<td><strong>24 h after CCl\textsubscript{4} treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>0.285 ± 0.044</td>
<td>322 ± 29</td>
<td>50.6 ± 2.7</td>
<td>2.78 ± 0.11</td>
</tr>
<tr>
<td>TJ-15 (100 mg/kg)</td>
<td>5</td>
<td>0.275 ± 0.031</td>
<td>319 ± 36</td>
<td>44.7 ± 3.2</td>
<td>2.82 ± 0.18</td>
</tr>
<tr>
<td>TJ-15 (250 mg/kg)</td>
<td>5</td>
<td>0.288 ± 0.021</td>
<td>333 ± 31</td>
<td>45.5 ± 8.1</td>
<td>2.91 ± 0.15</td>
</tr>
<tr>
<td>TJ-15 (500 mg/kg)</td>
<td>5</td>
<td>0.273 ± 0.047</td>
<td>288 ± 29</td>
<td>46.7 ± 6.2</td>
<td>2.95 ± 0.18</td>
</tr>
<tr>
<td>CCl\textsubscript{4}</td>
<td>14</td>
<td>0.795 ± 0.114*</td>
<td>475 ± 23*</td>
<td>20.2 ± 1.5*</td>
<td>1.21 ± 0.06*</td>
</tr>
<tr>
<td>CCl\textsubscript{4} + TJ-15 (100 mg/kg)</td>
<td>7</td>
<td>0.567 ± 0.065*#</td>
<td>436 ± 37*</td>
<td>28.1 ± 2.2*</td>
<td>1.97 ± 0.09*</td>
</tr>
<tr>
<td>CCl\textsubscript{4} + TJ-15 (250 mg/kg)</td>
<td>7</td>
<td>0.485 ± 0.083*#</td>
<td>389 ± 22*</td>
<td>41.1 ± 2.4*</td>
<td>2.31 ± 0.12*</td>
</tr>
<tr>
<td>CCl\textsubscript{4} + TJ-15 (500 mg/kg)</td>
<td>7</td>
<td>0.387 ± 0.041*#</td>
<td>345 ± 44*</td>
<td>45.1 ± 3.5#</td>
<td>2.55 ± 0.09*</td>
</tr>
</tbody>
</table>

Rats were intraperitoneally injected with either CCl\textsubscript{4} (1 m/kg body weight) or vehicle (olive oil) and then received orally either TJ-15 (100, 250 or 500 mg/kg body weight) or vehicle (distilled water) at 6 h after the toxicant treatment. The animals were sacrificed 6 or 24 h after CCl\textsubscript{4} treatment. Each value is a mean ± S.D. with the indicated number (N) of rats. *p<0.05 (vs. control group); \#p<0.05 (vs. CCl\textsubscript{4}-treated group).

Table 3. Effect of post-administered TJ-15 on hepatic changes in SOD, catalase, Se-GSH-px, GSSG-R, and G-6-PDH activities in CCl\textsubscript{4}-treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>SOD (µg/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>Se-GPX (mU/mg protein)</th>
<th>GSSG-R (mU/mg protein)</th>
<th>G-6-PDH (mU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6 h after CCl\textsubscript{4} treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>8.87 ± 0.61</td>
<td>232 ± 12</td>
<td>277 ± 12</td>
<td>4.14 ± 0.18</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>CCl\textsubscript{4}</td>
<td>5</td>
<td>7.38 ± 0.48*</td>
<td>251 ± 11</td>
<td>289 ± 28</td>
<td>3.98 ± 0.13</td>
<td>0.28 ± 0.05*</td>
</tr>
<tr>
<td><strong>24 h after CCl\textsubscript{4} treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>8.86 ± 0.57</td>
<td>247 ± 12</td>
<td>311 ± 13</td>
<td>4.25 ± 0.14</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>TJ-15 (100 mg/kg)</td>
<td>5</td>
<td>8.73 ± 0.82</td>
<td>251 ± 17</td>
<td>319 ± 14</td>
<td>4.28 ± 0.17</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>TJ-15 (100 mg/kg)</td>
<td>5</td>
<td>8.67 ± 0.98</td>
<td>246 ± 12</td>
<td>327 ± 26</td>
<td>4.32 ± 0.08</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>TJ-15 (100 mg/kg)</td>
<td>5</td>
<td>8.72 ± 0.62</td>
<td>245 ± 14</td>
<td>328 ± 32</td>
<td>4.25 ± 0.12</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>CCl\textsubscript{4}</td>
<td>14</td>
<td>4.52 ± 0.32*</td>
<td>145 ± 14*</td>
<td>279 ± 15</td>
<td>2.55 ± 0.06*</td>
<td>0.33 ± 0.04*</td>
</tr>
<tr>
<td>CCl\textsubscript{4} + TJ-15 (100 mg/kg)</td>
<td>7</td>
<td>6.63 ± 0.53#</td>
<td>178 ± 13#</td>
<td>281 ± 25</td>
<td>2.82 ± 0.12#</td>
<td>0.31 ± 0.03#</td>
</tr>
<tr>
<td>CCl\textsubscript{4} + TJ-15 (250 mg/kg)</td>
<td>7</td>
<td>7.88 ± 0.35#</td>
<td>191 ± 15#</td>
<td>295 ± 21</td>
<td>3.58 ± 0.22#</td>
<td>0.34 ± 0.04#</td>
</tr>
<tr>
<td>CCl\textsubscript{4} + TJ-15 (500 mg/kg)</td>
<td>7</td>
<td>8.46 ± 0.47#</td>
<td>225 ± 16#</td>
<td>297 ± 15</td>
<td>4.04 ± 0.11#</td>
<td>0.33 ± 0.05#</td>
</tr>
</tbody>
</table>

Rats were intraperitoneally injected with either CCl\textsubscript{4} (1 m/kg body weight) or vehicle (olive oil) and then received orally either TJ-15 (100, 250, or 500 mg/kg body weight) or vehicle (distilled water) at 6 h after the toxicant treatment. The animals were sacrificed 6 or 24 h after CCl\textsubscript{4} treatment. Each value is a mean ± S.D. with the indicated number (N) of rats. *p<0.05 (vs. control group); \#p<0.05 (vs. CCl\textsubscript{4}-treated group).

reduced progressive liver injury at 24 h in a dose-dependent
manner. In addition, this preventive effect of TJ-15 on
the progression of CCl\textsubscript{4}-induced liver injury was confirmed by
histological observation. Thus, orally administered TJ-15
was found to prevent the progression of CCl\textsubscript{4}-induced acute
liver injury in rats, as reported previously [9].

In the present study, rats treated once with CCl\textsubscript{4} had
increased hepatic TBARS concentration at 6 h after the
toxicant treatment and further increase in that concentration
at 24 h. In the CCl\textsubscript{4}-treated rats, hepatic GSH and ascorbic
acid concentrations and SOD activity decreased at 6 h after
the toxicant treatment and these decreases were enhanced at
24 h, while hepatic CAT and GSSG-R activities decreased at
24 h. However, the CCl\textsubscript{4}-treated rats showed an increase in

hepatic G-6-PDH activity and no change in hepatic Se-GPX activity at 6 and 24 h after the toxicant treatment. Hepatic XO activity in CCl4-treated rats increased 24 h after the toxicant treatment. These time-related changes observed in the liver of CCl4-treated rats were well consistent with those reported previously [9, 13, 14]. We have reported that XO-derived ROS contributes to stimulation of hepatic lipid peroxidation and disruption of hepatic antioxidant defense systems at a progressed stage of CCl4-induced acute liver injury in rats [13]. In addition, we have shown that the activity of Cu,Zn-SOD, which is localized in the cytosol of liver cells, but not Mn-SOD, which is localized in the mitochondria of liver cells, decreases in the liver of CCl4-treated rats [13, 14]. Our previous report has shown that a single oral administration of TJ-15 (500 mg/kg body weight) to CCl4-treated rats at an early stage of acute liver injury attenuates increased hepatic lipid peroxidation and decreased hepatic GSH level and SOD activity at a progressed stage of the injury [9]. In the present study, TJ-15 (100, 250 or 500 mg/kg body weight) administered orally to CCl4-treated rats at an early stage of acute liver injury attenuated the increase in hepatic TBARS concentration and XO activity and the decreases in hepatic GSH and ascorbic acid concentrations and SOD, CAT, and GSSG-R activities observed at a progressed stage of the injury in a dose-dependent manner. In addition, TJ-15 post-administered to CCl4-treated rats at a dose of 500 mg/kg body weight attenuated the increased hepatic XO activity and TBARS concentration and the decreased hepatic GSH concentration and SOD, CAT, and GSSG-R activities up to the levels of untreated control rats. However, no dose of post-administered TJ-15 had any effect on the hepatic Se-GPX activity and the increased hepatic G-6-PDH activity found at 24 h after CCl4 treatment. In addition, TJ-15 administered to CCl4-untreated rats at the same doses had no effect on the hepatic levels of these parameters studied. These results indicate that orally administered TJ-15 attenuates the disruption of hepatic ROS metabolism, in which antioxidants such as GSH and ascorbic acid, antioxidant enzymes such as SOD, CAT, and GSSG-R, and a pro-oxidant enzyme XO are involved, with the progression of CCl4-induced acute liver injury in rats. These results also suggest that this attenuating effect of TJ-15 on the disruption of hepatic ROS metabolism could contribute to its preventive effect on the progression of CCl4-induced acute liver injury. However, TJ-15 administered to CCl4-treated rats at a dose of 500 mg/kg body weight could not prevent the progression of acute liver injury completely, although the administered Chinese medicine could attenuate the disruption of hepatic ROS metabolism with the liver injury progression almost completely.

H2O2 and O2- inactivate Cu,Zn-SOD and CAT, respectively, in vitro [29, 30]. O2- and ˙OH, which is produced by the reaction of O2- and H2O2, inactivate GSSG-R in vitro [31, 32]. As described above, the decrease in SOD activity found in the liver of CCl4-treated rats is due to a decrease in the activity of Cu,Zn-SOD present in the tissue [13, 14]. TJ-15 scavenges O2- and ˙OH in vitro [2-4]. Therefore, one can assume that post-administered TJ-15 prevents the consumption of GSH and ascorbic acid and the inactivation of Cu,Zn-SOD, catalase, and GSSG-R due to ROS generated from increased XO in the liver of CCl4-treated rats by scavenging the generated ROS. Escobar et al. [33] have shown that alkyl-peroxyl radicals generated by 2,2'-azobis(2-amidinopropane), which is used as a radical source for lipid peroxidation, inactivate Cu,Zn-SOD and CAT. Accordingly, a possibility cannot be ruled out that post-administered TJ-15 protects Cu,Zn-SOD and CAT from peroxyl radical-mediated inactivation in the liver of CCl4-treated rats by inhibiting increased lipid peroxidation in the tissue.

It has been suggested that the CCl4-induced increase in hepatic XO activity might be due to the conversion of xanthine dehydrogenase (XD) to XO in the ischemic or hypoxic liver [12]. The conversion of XD to XO in liver tissues is caused by a proteolytic mechanism and/or the oxidation of sulfhydryl groups present in the protein [34, 35]. Therefore, post-administered TJ-15 may prevent the oxidation of sulfhydryl groups present in the protein of XD in the liver of CCl4-treated rats through its antioxidant action, resulting in inhibition of the conversion of XD to XO in the tissue.

TJ-15 is prepared with the boiled water extract of a mixture of four herbs, i.e., Coptidis Rhizoma, Scutellariae Radix, Phellodendri Cortex, and Gardeniae Fructus. Miao et al. [36] reported that when Coptidis Rhizoma, Scutellariae Radix, and Phellodendri Cortex were extracted with 50% methanol, the Scutellariae Radix extract had much higher O2- -scavenging activity than the Phellodendri Cortex extract, while the Coptidis Rhizoma extract had little activity. It is known that among the boiled water extracts of Coptidis Rhizoma, Gardeniae Fructus, Scutellariae Radis, and Phellodendri Cortex, the Coptidis Rhizoma extract has the highest inhibitory activity on lipid peroxidation induced by H2O2: in rat liver homogenates [37]. It is also known that the boiled water extract of Coptidis Rhizoma, Scutellariae Radix or Phellodendri Cortex, but not Gardeniae Fructus, inhibits lipid peroxidation induced by H2O2 plus FeSO4, i.e., ˙OH generated from the so-called Fenton reaction, in rat liver homogenates [37]. Baicalein, baicalin, and wogonin,
which are present in Scutellariae Radix, scavenge OH' and alkyl radical in vitro [38]. Berberine present in Coptidis Rhizoma and Phellodendri Cortex also scavenge O2−, but not ‘OH, in vitro [39]. These findings may allow us to assume that various components present in the four constituent herbs of TJ-15 contribute to the attenuating effect of the Chinese medicine on the disruption of hepatic ROS metabolism with the progression of CCl4-induced acute liver injury in rats.

In conclusion, the results of the present study indicate that orally administered TJ-15 attenuates the disruption of hepatic ROS metabolism, in which GSH, ascorbic acid, SOD, CAT, GSSG-R, and XO are involved, with the progression of CCl4-induced acute liver injury in rats through its direct and indirect antioxidant actions, dose-dependently. These results also suggest that this attenuation by TJ-15 of the disruption of hepatic ROS metabolism could contribute to its preventive effect on the progression of CCl4-induced acute liver injury.

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