Treatment of Kangxian Pills to Chronic Liver Injury in Mice Induced with Carbon Tetrachloride

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ABSTRACT

Objective Kangxian Pills, containing Angelicae Sinensis, Chuanxiong Rhizoma, radix paeoniae rubra, and 12 other kinds of Chinese materia medica, have the functions of softening and dispersing blood stasis. It has been used for liver injury and liver fibrosis. The current study was designed to evaluate the anti-hepatic injury activity and the mechanism of Kangxian Pills on a CCl₄-induced animal model. Methods To induce chronic liver injury, mice were treated with CCl₄ twice a week for four weeks. Kangxian Pills (6 or 12 g/kg) and Compound Biejia Ruangan Tablet (0.901 g/kg) were ig given to mice once daily for four weeks after CCl₄ was withdrawal. The anti-hepatic injury activities and mechanisms of Kangxian Pills were assessed by hepatic histology and by measuring the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), and total protein (TP) of serum as well as superoxide dismutase (SOD) and glycogen (Gn) of the liver. Results Kangxian Pills significantly decreased the levels of liver index, ALT, and AST in mice liver injury models in treatment group. Moreover, Kangxian Pills and Compound Biejia Ruangan Tablet inhibited the CCl₄-induced reduction of SOD and Gn levels in the liver. The histological study showed that Kangxian Pills could reduce cellular swelling and infiltration of inflammatory cells in liver injury. Conclusion Kangxian Pills possess the potent abilities to alleviate chronic liver injury, suggesting that Kangxian Pills exert this effect by enhancing the anti-oxidant ability and metabolism of the liver.

Key words
Angelica sinensis; carbon tetrachloride; Kangxian Pills; Ligusticum wallichii; liver injury

1. Introduction

Liver injury can be induced directly from hepatotoxicity or indirectly from immune mediation by biological factors (hepatitis virus, bacteria, parasite, etc.), chemical factors (medicine, industrial poisons, alcohol, etc.) and environmental factors (Holt and Ju, 2006). These factors would induce apoptosis and necrosis of hepatic cells (Wang, 2014; Friedman et al, 1985; Maher and McGuire, 1990). Long term repeated hepatic cells necrosis leads to hepatic fibrosis, liver cirrhosis, and even hepatocellular carcinoma. Therefore, the prevention of liver injury is a critical step for protecting liver

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against the occurrence of cirrhosis and liver function failure.

Chinese materia medica (CMM) has been universally practiced in China for thousands of years and has been widely used in the treatment of chronic disease based on less side-effects and high efficiency. The species in Astragalus Linn. have been used in the prevention and treatment of side-effects and high efficiency. The species in used in the treatment of chronic disease based on less

The aim of this study was to investigate the effect of Kangxian Pills of Kangxian Pills preventing liver injury remains unknown. The mechanisms for the treatment of liver injury and liver fibrosis, the mechanism in Kangxian Pills preventing liver injury remains unknown. The aim of this study was to investigate the effect of Kangxian Pills on the prevention of chronic liver injury induced by CCl4 in mice and evaluate the possible mechanism of anti-hepatic injury. Compound Biejia Ruangan Tablet, a commonly used anti-hepatic injury medicine, was used as a positive control. The anti-hepatic injury activities of Kangxian Pills were assessed by hepatic histology and by measuring levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), and total protein (TP) of serum, which are frequently-used parameters of liver function. Moreover, superoxide dismutase (SOD) and glycogen (Gn) of mice were investigated in an effort to elucidate the possible mechanisms by which Kangxian Pills exert their hepatic protective activity.

2. Materials and methods

2.1 Animal and reagent

ICR mice were purchased from Beijing HFK Bioscience Company; Kangxian Pills were purchased from Tianjin Second People’s Hospital; Compound Biejia Ruangan Tablet was purchased from Nei Monggol Furuizhong Drug Science Company; Carbon tetrachloride (CCl4), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), total protein (TP), superoxide dismutase (SOD), and glycogen (Gn) test kits were purchased from Nanjing Jiancheng Biological Engineering Institute.

2.2 Methods

2.2.1 Animals and treatments

Mice were housed at room temperature of (23 ± 1) °C with a 12 h light-12 h dark cycle (lights on from 6:00 am to 6:00 pm). Food and water were available ad libitum. The experiments were carried out according to the institutional regulations and national criteria for animal experimentation. The Institution Animal Ethics Committee reviewed the entire animal protocol prior to conducting the experiments.

Mice were randomly divided into five groups: Group A: control group (n = 6); Group B: CCl4 model group (n = 10); Group C: positive control group (n = 10); Group D: low-dose Kangxian Pills group (n = 10); Group E: high-dose Kangxian Pills group (n = 10).

After 3 d of acclimatization, chronic liver injury was induced as described previously (Ohta and Sahashi, 2002). In brief, the mice in Groups B–E received ip injection of 20% CCl4 solution diluted in olive oil (2 mL/kg body weight), twice weekly for a four-week period. The mice in Group A received an ip injection of olive oil (2 mL/kg body weight) instead of CCl4. After four weeks when CCl4 was withdrawal, the mice in Groups A and B received normal saline orally for four weeks daily, and the mice in Group C were given Compound Biejia Ruangan Tablets (0.901 g/kg body weight) diluted in normal saline orally for four weeks daily. The mice in Groups D and E received Kangxian Pills (6 and 12 g/kg body weight, respectively) orally for four weeks daily.

At the end of the four weeks Compound Biejia Ruangan Tablets and Kangxian Pills administration, mice were anesthetized with ether and blood was obtained from the retrobulbar plexus for serum biochemistry analysis. Blood samples were centrifuged at 3000 r/min for 15 min and serum was collected and kept at –80 °C for analysis.

Animals were then decapitated; Livers and spleens were removed and weighed. Half of the livers were then fixed in 10% formalin for histological analysis, others were stored at –80 °C for anti-oxidant analysis.

2.2.2 Serum and tissue analysis

ALT, AST, ALB, TP in serum, and SOD and Gn in liver were measured on a Plate Reader using diagnostic reagent kit.

2.2.3 Histological analysis

Liver tissue sections were dissected and fixed in 10% formalin, then embedded in paraffin, sectioned to 5 μm thickness, and stained with hematoxylin and eosin (H&E). The extent of CCl4-induced liver injury was evaluated by assessing morphological changes in liver sections.

2.2.4 Statistics

Quantitative data were expressed as $\bar{x} \pm s$ and compared using ANOVAK independent samples test when variances were heterogeneous. Data were considered significant difference when $P < 0.05$.

3. Results

3.1 General statement

The initial body weight was not significantly different in the five groups (Figure 1a). The body weight of mice treated
with CCl₄ was significantly lower than that of mice treated with olive oil for the same period ($P < 0.01$). The body weights in Groups C–E were significantly higher compared with Group B ($P < 0.05$, Figure 1b). Liver index in Group B was significantly higher than that in Group A ($P < 0.01$). Groups C–E had significantly lower liver index than Group B ($P < 0.05$, 0.01, 0.05, respectively, Figure 2). The abdominal girth of mouse was measured to investigate the statement of abdominal dropsy. The abdominal girth was bigger in Group B than that in Group A ($P < 0.05$). The abdominal girth in Groups C–E were significantly decreased compared with Group B ($P < 0.05$, Figure 3).

3.2 Changes of indexes in serum

Administration of CCl₄ for four weeks caused the significant elevation of ALT and AST activities in mice serum compared with mice treated with olive oil ($P < 0.01$) and there were no difference in ALB and TP activities between the mice treated with CCl₄ and olive oil ($P > 0.05$, Table 1). After four-week administration of Compound Biejia Ruangan Tablet and Kangxian Pills, the increased ALT and AST activities remained significantly higher in Group B than those in Group A ($P < 0.01$). Serum AST activity was significantly lower in Group D than that in Group B ($P < 0.01$). Groups C–E demonstrated a significant decrease in ALT levels compared to Group B ($P < 0.05$, 0.01, 0.05, respectively). Levels of ALB and TP showed no difference in Groups C–E compared with Group B ($P > 0.05$, Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U·L⁻¹)</th>
<th>AST (U·L⁻¹)</th>
<th>ALB (U·L⁻¹)</th>
<th>TP (U·L⁻¹)</th>
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<tbody>
<tr>
<td>Group A (n = 6)</td>
<td>28.64 ± 9.59</td>
<td>21.20 ± 10.09</td>
<td>29.46 ± 3.29</td>
<td>55.27 ± 5.54</td>
</tr>
<tr>
<td>Group B, C, D and E (n = 40)</td>
<td>75.61 ± 11.81</td>
<td>79.48 ± 8.20</td>
<td>55.19 ± 3.58</td>
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$^{**}P < 0.01$ vs Group A; $^*P < 0.05$ vs Group B

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U·L⁻¹)</th>
<th>AST (U·L⁻¹)</th>
<th>ALB (U·L⁻¹)</th>
<th>TP (U·L⁻¹)</th>
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<tr>
<td>A (n = 6)</td>
<td>41.40 ± 19.81</td>
<td>27.36 ± 13.42</td>
<td>37.46 ± 6.87</td>
<td>61.07 ± 6.74</td>
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<tr>
<td>B (n = 10)</td>
<td>75.72 ± 19.26 $^{**}$</td>
<td>83.28 ± 20.65 $^{**}$</td>
<td>58.44 ± 6.62</td>
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<tr>
<td>C (n = 10)</td>
<td>67.86 ± 26.21</td>
<td>34.64 ± 30.76 $^{**}$</td>
<td>59.19 ± 5.81</td>
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<tr>
<td>D (n = 10)</td>
<td>39.33 ± 20.17 $^{**}$</td>
<td>54.35 ± 30.20 $^{**}$</td>
<td>59.02 ± 4.13</td>
<td></td>
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<tr>
<td>E (n = 10)</td>
<td>68.72 ± 13.49</td>
<td>55.40 ± 8.73 $^{**}$</td>
<td>58.49 ± 5.37</td>
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$^{**}P < 0.01$ vs Group A; $^{*}P < 0.05$ vs Group B $^{**}P < 0.01$ vs Group B
3.3 Changes of indexes in liver tissue

Activity of SOD was significantly lower in Group B than that in Group A \((P < 0.01)\); Groups C–E demonstrated an increase in SOD activities compared with Group B, the difference was statistically significant \((P < 0.05, \text{Figure 4})\). The level of liver glycogen was higher in Groups C and D than that in Group B \((P < 0.05, \text{Figure 5})\).

3.4 Histological findings

CCl\(_4\) treatment induced a significant amount of liver injury and extensive changes in liver morphology, including the infiltration of inflammatory cells and obvious cellular swelling of the liver, as observed with HE staining. Liver injury was attenuated in Groups C–E after four weeks. Most importantly, pathological changes in Groups D and E were slighter than those in Group B (Figure 6).

4. Discussion

In this study, an animal model of CCl\(_4\)-induced chronic liver injury was established to examine the effects of Kangxian Pills on a chronic liver injury model by the study of many frequently used clinical parameters. A CCl\(_4\)-induced liver injury, which has many aspects in common with human liver injury, is a common model for studying the mechanisms of liver injury and the therapeutic effects of drugs (Fort et al., 1998; Tamayo, 1983). In the liver, CCl\(_4\)-inhibited metabolism and activated hepatic cytochrome P450s were used to produce trichloromethyl radicals CCl\(_3\) and OOCCl\(_3\). These free radicals contribute to lipid peroxidation to disrupt calcium homeostasis, gradually leading to the necrosis of hepatocytes and cell membrane injury surrounding the central vein. Finally, the necrosis of hepatocytes and hypo-immunity result in liver injury (Wang et al., 2010; Xu and Qu, 2008; Bockhold et al., 2005; Nelson, 2006). Thus, this model may mimic the morphology and pathophysiology of hepatocyte regeneration after necrosis in humans, as well as other abnormal signs and changes in liver function (Friedman and Bio, 2000).

AST, ALT, ALB, and TP, which are conventional indicators of chronic liver injury, were measured to investigate the hepatic protective effect of Kangxian Pills. The present study revealed continuing, changed the levels of AST and ALT in the CCl\(_4\) model group at the end of the
fourth week, indicating considerable hepatocellular injury. The administration of Kangxian Pills (6 or 12 mg/kg) for four weeks improved the levels of ALT and AST in serum.

In addition, the probable mechanism of Kangxian Pills preventing chronic liver injury may increase the levels of SOD and glycogen in the liver. SOD is the exclusive enzyme which eliminates the generation of free radicals (O$_2^-$) in order to relieve the damage of trichloromethyl radicals in the liver (Wang et al., 2011). Glycogen is a kind of energy reserve in the liver and muscles. Some hepatic protective medicine such as Swertiae Mussotll Herba and lutein can increase the liver glycogen to prevent liver injury (Ding et al., 2007; Zhang et al., 2009). So, the increase in liver glycogen may be one of the pathways preventing chronic liver injury of Kangxian Pills. Compared with Compound Biejia Ruangan Tablet, Kangxian Pills have better effects in changing the serum index of the liver and reducing the edema in liver cells.

Moreover, recently, studies have demonstrated that the combination of some CMM and Western herbs is more effective for some chronic diseases. Reports have shown that some CMM such as Compound Biejia Ruangan Tablet (He and Xu, 2010) and Kushenin (Su et al., 2010) combined with adefovir dipivoxil exhibit more efficacies on the treatment of liver fibrosis and chronic hepatitis. So, further study is required to elucidate the effect of the combination of Kangxian Pills and Western medicine.

References


