Hepatoprotective effect of manual acupuncture at acupoint GB34 against CCl\textsubscript{4}-induced chronic liver damage in rats

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Abstract

AIM: To investigate the hepatoprotective effect of manual acupuncture at Yanglingquan (GB34) on CCl\textsubscript{4}-induced chronic liver damage in rats.

METHODS: Rats were injected intraperitoneally with CCl\textsubscript{4} (1 mL/kg) and treated with manual acupuncture using reinforcing manipulation techniques at left GB34 (Yanglingquan) 3 times a week for 10 wk. A non-acupuncture group in left gluteal area was selected as a sham point.

RESULTS: Manual acupuncture at GB34 reduced the liver index, serum ALT, AST, ALP and total cholesterol, histological analysis and blood cell counts were conducted.

CONCLUSION: Manual acupuncture with reinforcing manipulation techniques at left GB34 reduces liver toxicity, protects liver function and liver tissue, and normalizes immune activity in CCl\textsubscript{4}-intoxicated rats.

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Key words: Manual acupuncture; Yanglingquan (GB34); CCl\textsubscript{4}-induced liver damage; Hepatoprotective effect

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INTRODUCTION

Herbal medicine and acupuncture are the two main methods to treat disease in oriental medicine. Because of chemical residue contamination, there is recent gaining suspicion that herbs may be harmful to the liver. Accordingly, acupuncture is getting more interest these days for the treatment of liver diseases in oriental medical clinics.

In the present study, we tried to investigate the effects of manual acupuncture on long-term liver damage. To investigate the effects of manual acupuncture at GB34 on liver damage, we used CCl\textsubscript{4}-intoxicated rat model and chose GB34 as an acupoint to protect liver and treat liver damage induced by CCl\textsubscript{4} administration.

GB34 is an acupoint located on the gall bladder meridian. In oriental medical theory, the liver and gall bladder corresponds to each other and their meridians are also closely related with each other in the ‘interior-exterior relationship’\textsuperscript{1,2}. Gall bladder meridian pertains to the gall bladder organ and connects with the liver organ. The liver meridian pertains to the liver organ and connects with the gall bladder organ. Therefore, the acupoints on the liver meridian are used to treat gall bladder organ diseases as well as liver organ diseases. Consequently, the acupoints on the gall bladder meridian are used to treat liver organ diseases as well as gall bladder organ diseases. Hence, GB34 is closely related with the liver as well as the gall bladder. It explains why GB34 is used to treat liver disease so often. Moreover, GB34 is the ‘He’ (meaning ‘sea’) point of gall bladder. In oriental medical theory, a ‘sea He’ point is considered to be the entrance of the meridian energy to the corresponding organ\textsuperscript{3}. Therefore, GB34 influences the liver and gall bladder more strongly than other acupoints. The functions of this point are regulating and tonifying the liver, regulating the gallbladder, spreading liver Qi (oriental medical term for “vital energy”), subduing liver Yang, draining liver pathogens, etc. GB34 has been clinically used for hypochondriac pain, jaundice, hepatitis, acute biliary tract diseases, cirrhosis of the liver and hypertension due to liver Yang excess, etc\textsuperscript{4}.

We presume that neuronal activity is involved in the transmission of acupuncture stimulation, so the animals were not anesthetized during the acupuncture administration.
tion. To keep the animals from moving during the acupuncture administration, they were put in cages with five holes for tail and four limbs. To estimate and exclude the effect of stress from restriction within the cage, the rats in the control group were also kept in the cages in the same manner as the acupuncture group.

The action of acupuncture could be influenced by acupuncture techniques as well as point selection. There are two main categories of acupuncture techniques: reinforcing technique and reducing technique. Clockwise needle rotation, scraping downward of needles, and odd number of manipulating operations are considered as reducing techniques. On the other hand, counterclockwise needle rotation, scraping upward of needles, and even number of manipulating operations are considered as reinforcing techniques. Reinforcing acupuncture manipulation techniques are used for chronic and deficient syndrome, while reducing techniques are used for acute and excess syndrome. Since the animals in the present study were injected with CCl4 for a long period, we considered it as a chronic and deficient condition, and therefore, we administered acupuncture with reinforcing manipulation techniques.

MATERIALS AND METHODS

Animals
Sprague-Dawley male rats (200-250 g) were purchased from Deahan Biorink Co. The animals were adapted to the environment of 22 ± 2°C room temperature, 12-h light/dark cycle for 2 wk and had free access to water and food. Our animal experiment has been conducted in accordance with the Use of Laboratory Animals as adopted by the U.S. National Institutes of Health.

Experimental design
Experimental animals were randomly divided into four groups: normal; control (CCl4); Sham (CCl4 + manual acupuncture at sham point); and GB34 (CCl4 + manual acupuncture at left GB34). Each group consisted of 7 rats. Liver injury was induced by intraperitoneal injections with 500 mL/L CCl4 (Sigma, USA) solution in olive oil (1 mL/kg), twice a week for 10 wk. Manual acupuncture was administered 3 times a week during the same period.

Liver index measurement
Rat’s body weight was measured before the animals were sacrificed. Rat liver was removed and weighed right after the animal was sacrificed and the liver index (% of liver weight/body weight) was estimated.

Biochemical analysis and blood counts
Forty-eight hours after the last administration with CCl4, the rats were anesthetized with ethyl ether and blood samples were taken from the heart. Blood was centrifuged at 3000 r/min for 15 min and serum was taken. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphotase (ALP), total cholesterol in serum and the populations of RBC, WBC, lymphocytes in plasma were detected.

Histological analysis
The rats were sacrificed and the liver tissues were obtained individually from each group and fixed in 40 g/L formaldehyde. After decalcification in 50 mL/L formic acid, the specimens were processed for paraffin embedding. Tissue sections were obtained and stained with hematoxylin and eosin (HE) or masson’s trichrome (MT). Tissue destruction and fatty changes of liver were observed at 400 × magnification.
Statistical analysis
Data were obtained from the rats which survived to the end of the experiment. All in normal group, 3 in control group, 3 in sham group and 5 in GB34 group survived to the end of the experiment. Data were expressed as mean ± SD. Statistical significance of difference between groups was determined using ANOVA, followed by t-test. \( P < 0.05 \) was considered statistically significant.

RESULTS
Liver index
CCl\(_4\) injection induced a significant increase in liver index. On the other hand, manual acupuncture at GB34 lowered it similar to the normal value (Figure 3).

Serum ALT, AST, ALP and total cholesterol
ALT, AST, ALP and total cholesterol in serum were increased remarkably by long-term CCl\(_4\) administration, indicating damage to the liver. Manual acupuncture at GB34 significantly reduced serum ALT, AST and total cholesterol in comparison with the control group. Serum ALP was also reduced by manual acupuncture at GB34 but no statistical significance was found (Figure 4).

Blood cell counts
The number of RBC was slightly reduced by CCl\(_4\) intoxication and restored by manual acupuncture at GB34, though statistical significance was not observed. The number of WBC and the percentage of lymphocytes out of WBC was significantly reduced by CCl\(_4\) intoxication and restored by manual acupuncture at GB34 close to the normal level (Figure 5).

Liver histology
Histological analysis using HE stain showed that necrosis of liver tissue and fatty changes were viciously induced by CCl\(_4\) administration. The group treated with manual acupuncture at GB34 showed reduced feature of hepatocyte necrosis and fatty change compared to the control group and the sham group. In addition, MT staining showed lower accumulation of extracellular matrix in the GB34 group compared to the control group and sham group (Figure 6).

DISCUSSION
CCl\(_4\) has been widely used to induce experimental hepatic...
In the present study, long-term CCl\textsubscript{4} administration reduced the population of leucocytes and lymphocytes in blood. We infer that this reduction was due to the decline of immune activity by long-term liver damage. Manual acupuncture at GB34 recovered the population of leucocytes and lymphocytes in blood. Therefore, we presume that manual acupuncture at GB34 restored the immune activity, which is also in agreement with Hau’s report on the anti-fibrotic effect of manual acupuncture at GB34 protected liver and reduced liver toxicity. Histological analysis in this study was only qualitative. Quantitative assessment of the amount of hepatic hydroxyproline content would be meaningful in the next study.

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Based on the results of the present study, we speculate that manual acupuncture at GB34 is beneficial to protect liver function and tissue, reduce hepatic toxicity and normalize immune activity against CCl\textsubscript{4}-intoxication in rats. The hepatoprotective effect of manual acupuncture at GB34 in this study may be related to the immune reinforcing effect of acupuncture or neuro-immune interaction on the pathway of the transmission of acupuncture stimulation.

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