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Protective effect of artemisinin on chronic alcohol induced-liver damage in mice

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ABSTRACT

The liver disease related to chronic alcohol consumption is one of the leading causes of death for alcoholics. The efficient drug to ameliorate the alcoholic liver injury was needed urgently. The present study was performed to investigate whether artemisinin possessed the protective effect against chronic alcohol consumption. 50 male Kunming mice were divided into 5 groups: control group (C): 10 ml/kg saline + 10 ml/kg saline, alcohol group (A): 10 ml/kg 56%(v/v) alcohol + 10 ml/kg saline, low dose group of artemisinin (L): 10 ml/kg 56%(v/v) alcohol + 30 mg/kg/day artemisinin, medium dose group of artemisinin (M): 10 ml/kg 56%(v/v) alcohol + 60 mg/kg/day artemisinin, high dose group of artemisinin (H): 10 ml/kg 56%(v/v) alcohol + 120 mg/kg/day artemisinin. Drugs were given orally every day. The general state of mice was observed and the levels of serum activities of AST and ALT were detected after treatment with drugs for 30 days. Besides, the liver weight index was calculated and histopathological analysis was performed. We successfully demonstrated that treatment with high dose of artemisinin significantly decreased the elevated levels of AST ($p < 0.05$) and ALT ($p < 0.01$) in plasma, as well as the liver weight index ($p < 0.01$). The loss of body weight, tissue injury, oedema and inflammatory cell infiltration in the hepatocytes were found in the A group. These symptoms were remarkably alleviated in animals treated with artemisinin. Artemisinin can inhibit the activation of NF-κB and the expression of inflammatory cytokines inducible nitric oxide synthase. Besides, it can also enhance the stability of liver cell membrane, and reduce the damage of liver cell membrane and liver cell. Artemisinin showed a protective effect against chronic alcohol poisoning and it has a great potential for the clinical application to treat the liver injury induced by alcohol.

1. Introduction

Alcoholism, a severe worldwide health problem, has caused many disorders including alcoholic fatty liver, alcoholic hepatitis, alcoholic fibrosis and alcoholic cirrhosis (Rong et al., 2012). It has been suggested that the liver could be severely damaged due to chronic alcohol intake (Kundua et al., 2008). The possible mechanisms of the alcohol-induced oxidative stress have been reported, including the change in redox state, production of acetaldehyde, mitochondrial injury, the mobilization of iron and the decrease of antioxidant enzymes (Giriwono et al., 2010). The oxidative stress and the generation of free radicals play critical roles in chronic alcohol induced-liver damage (Wang et al., 2012). More phenolic hydroxyl groups with hydrogen donor activity have been found which can inhibit the primary reaction of free radicals. It was also reported that the complement system showed a protective effect against alcohol-induced rat liver damage (Bykov et al., 2004). However, the clear pathogenetic mechanisms are still poorly understood.

Previous studies have shown that some natural plant products, such as Curcumin and Puerarin, possessed protective effects against the liver disorder induced by chronic alcohol intake. The main sources of biologically active compounds come from the natural plant products. Thus, the natural products with antioxidant activity become more and more attracted.

Artemisinin, isolated from the compositae plants Artemisia annua, is a sesquiterpene lactone secondary metabolite with an endoperoxide

Abbreviations: C, control group; A, alcohol group; L, low dose group of artemisinin; M, medium dose group of artemisinin; H, high dose group of artemisinin; ALT, alanine aminotransferase; AST, aspartate transaminase; NF-κB, nuclear factor κB

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bridge (Kim et al., 2008). Artemisinin and its analogs have been used to treat malaria (Laia et al., 2005). In addition, it has been investigated that artemisinin possessed the potent anti-cancer activity in vitro (Singh and Lai, 2001; Singh and Lai, 2004; Efferth et al., 2004) and in vivo (Moore et al., 1995; Chen et al., 2004; Lai and Singh, 2006). Besides, it has been shown that artemisinin possessed the potential of anti-fibrosis and anti-inflammatory effects (Wong and Menendez, 1999). The possible effect of artemisinin in the regulation of inflammatory responses has been demonstrated (Tan et al., 1999).

However, no study has been carried out concerning the protective effects of artemisinin on chronic alcohol induced-liver damage yet. The present study was performed to investigate the possible effect of artemisinin on chronic alcohol induced-liver damage in mice. The results showed that artemisinin significantly ameliorated chronic alcohol-induced structural abnormalities in liver tissue and the serum levels AST and ALT.

2. Materials and methods

2.1. Drugs and reagents

Artemisinin (Purity ≥ 99.5%, Molecular weight: 282.34, Cas number: 63968-64-9) was obtained from Aladdin chemistry Co. Ltd (Shanghai, China). The alcohol was purchased from Hongxing Co. Ltd (Beijing, China). The alanine aminotransferase (ALT) and aspartate transaminase (AST) reagent kit were purchased from Jiancheng Bioengineering Institute (Nanjing, China). The arowana edible oil was purchased from Wilmar International Limited (Shenzhen, China).

2.2. Experimental animals

A total of 50 male KM mice (about 16–18 g) were maintained at a controlled ambient temperature (23 ± 2 °C) under diurnal conditions. All mice were allowed free access to food and water. The mice were purchased from Animal Center (SCXK 2009-0001), Chongqing University of Medicine, Chongqing, China. The study was strictly carried out according to the recommendations in the Guide for the Laboratory Animal Care and Use of the National Institutes of Health. And the experiments were approved by the Ethical Committee for Animal of Southwest University (Permit Number: SYXK 2009-0002).

2.3. Experimental protocal

The mice were randomly divided into five groups with 10 mice in each group: control group (C), alcohol group (A), low dose group of artemisinin (L) (30 mg/kg/day), medium dose group of artemisinin (M) (60 mg/kg/day) and high dose group of artemisinin (H) (120 mg/kg/day). The detailed treatments are as follows: first, mice in C group were given saline (10 ml/kg), in the other four groups were given 10 ml/kg 56%(v/v)alcohol. After 30 min, the animals in C and A groups were given saline (10 ml/kg), L group were given artemisinin (30 mg/kg/day), M group were given artemisinin (60 mg/kg/day), and mice in H group were given artemisinin (120 mg/kg/day). The dose of artemisinin was selected to be 120 mg/kg/day to 30 mg/kg/day according to the previous study. Artemisinin was dissolved in arowana edible oil (6 mg/ml). We also set up an arowana edible oil group and found no adverse or beneficial effect at this dose (data not shown). All drugs were given orally. During the experiment, animals were maintained under standard conditions with free access to food and water for 30 days. In the end, blood samples were collected and the levels of serum AST and ALT were determined. And then, all animals were killed and the livers were excised, weighed and fixed in 4% formaldehyde. Histopathological analysis was performed.

2.4. Behavior study and general toxicity

Food and water were provided during the experiment, and behavioral changes of the mice were observed every day. Besides, body weight was recorded weekly until the end of the experiment.

2.5. Determination of plasma aspartate transaminase (AST)

AST activity is a very sensitive indicator to evaluate the injury of liver. The blood samples were collected from the intraocular angular vein and then the serum was prepared. Then the serum activity of AST was measured with a commercial kit purchased from Jiancheng Bioengineering Institute (Nanjing, China), referring to the manufacturer's instructions.

2.6. Determination of plasma alanine aminotransferase (ALT)

The level of serum ALT activity is another indicator to show the liver injury. So we used a Detection Kit to measure the ALT activity, according to the manufacturer's protocol.

2.7. Histopathological analysis

At the end of the experiment, liver biopsies were removed and fixed in 4% formaldehyde, embedded in paraffin, sectioned, and then stained with haematoxylin/eosin. The light microscopy was used to study the morphological changes in the experimental mice. We establish grades to evaluate the degree of the hepatocyte necrosis and inflammatory infiltrate: grade 0 (no pathological change), grade 1 (minimal hepatocyte necrosis and inflammatory infiltrate), grade 2 (mild), grade 3 (moderate), grade 4 (severe inflammatory cells infiltration).

2.8. Statistical analysis

All data are presented as mean ± SEM. Statistical significance of differences between groups was performed with Student's *t*-test or one-way ANOVA followed by post hoc analysis. $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed with SPSS Statistical Software (version 16.0 for Windows).

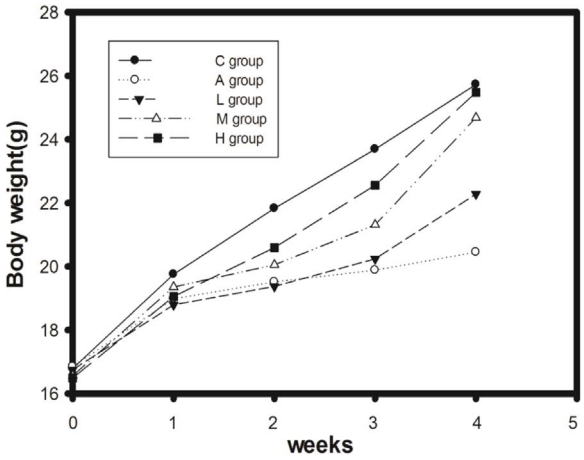

Fig. 1. Effect of artemisinin on body weight changes in chronic alcohol induced-liver damage in mice.Body weight were measured weekly during the course of experiment. The result showed that the A group were in poor health compared to the other groups.