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Preliminary report

The hepatoprotective effect of fraxetin on carbon tetrachloride induced hepatic fibrosis by antioxidative activities in rats

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ABSTRACT

The aim of the study was to investigate the potentially protective effects of fraxetin on carbon tetrachloride (CCl_4) induced oxidative stress and hepatic fibrosis in Sprague–Dawley rats. In this study, rats were divided into five groups, including normal controls, model, silymarin as the positive control, fraxetin 20 mg/kg and fraxetin 50 mg/kg. After 8 weeks, activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBIL) were checked. The levels of protein carbonyls, thiobarbituric acid-reactive substances (TBARS) and antioxidant enzymes such as catalase, SOD and glutathione peroxidase (GSH-Px) were determined after fraxetin administration. The hydroxyproline levels and histopathologic examinations of hepatocyte fibrosis were also determined. We found that fraxetin at doses of 20 and 50 mg/kg for 8 weeks significantly reduced the levels of TBARS and protein carbonyls compared with CCl_4 group. Fraxetin significantly increased the activities of catalase, SOD and GSH-Px in the liver. We also found that fraxetin prevented CCl_4 induced hepatic fibrosis by histological observations. These results indicate that fraxetin exhibits potent protective effects against CCl_4 induced oxidative stress and hepatic fibrosis.

1. Introduction

Hepatic fibrosis is a common pathological process resulted from various chronic hepatic injuries, which is characterized by an increase of extracelluarmatrix (ECM) deposition in the Disse's space and the imbalance between synthesis and degradation of ECM. Accumulating evidence suggests that hepatic fibrosis is a reversible disease, therefore an effective treatment would probably prevent or reverse the hepatic fibrotic process [1]. In recent years, considerably clinical and experimental evidences show that oxidative stress caused by an imbalance between the oxidant and antioxidant systems of the body in favor of the oxidants should be a major apoptotic stimulus in the different types of acute and chronic liver injury and hepatic fibrosis [2]. CCl_4 is a potent hepatoxin producing centrilobular hepatic necrosis which is widely used for animal models of liver fibrosis. Hepatic fibrosis induced by CCl_4 is associated with the exacerbation of lipid peroxidation and the depletion of antioxidant status [3]. Accordingly, successful antioxidant interventions may be a potential and effective therapeutic strategy for prevention and treatment of hepatic fibrosis.

Coumarins comprised a group of phenolic compounds widely distributed in natural plants, such as citrus fruits, tomatoes, vegetables, and green tea. Their popularity has increased because of the range of pharmacological properties demonstrated, such as antithrombotic [4], anti-inflammatory [5], antiviral [6], and antitumor properties [7]. Fraxetin (7,8-dihydroxy-6-methoxy coumarin), a coumarin derivative, has been reported to possess antioxidative, anti-inflammatory and neuroprotective effects [8–11]. Fraxetin exhibits its antioxidant effect through increasing the level of GSH and reducing oxidative damage in a drosophila melanogaster experimental model [8]. In addition, previous studies reported that some coumarin derivatives include fraxetin, was able to protect neuroblastoma cells against toxic effects induced by rotenone [10,11].

Because of the strong antioxidant activity of fraxetin, we hypothesised that fraxetin administration might be useful for preventing various types of oxidative damage induced by oxidative stress. Therefore, the aims of this study were to evaluate the antioxidant and antifibrotic properties of fraxetin in vivo.

2. Materials and methods

2.1. Chemicals

Carbon tetrachloride (CCl_4), Fraxetin (7,8-dihydroxy-6-methoxy coumarin),and silymarin were obtained from Sigma Chemicals Company.

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Corn oil was purchased from the local market, sealed and stored at room temperature after high temperature sterilization.

2.2. Animals

Male Sprague–Dawley rats (150–200 g) were obtained from Wenzhou Medical College. The rats were housed under normal laboratory conditions (21 ± 2°C, 12/12 h light-dark cycle) with free access to standard pellet diet and water ad libitum. All animal procedures were conducted in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the Committee for the Purpose of Control and Supervision of Experiments on Animals and the National Institutes of Health. The study protocol was approved by the Animal Ethics Committee of Wenzhou Medical Collage.

2.3. Treatment

The animals were randomly divided into 5 groups of 7. Group I served as the normal control and was orally administered distilled water daily with intraperitoneally (i.p.) administered corn oil (1 ml/kg body weight), which twice per week for 8 weeks. To induce oxidative stress and hepatic fibrosis, animals of Groups II, III, IV and V were i.p. administered 2 ml/kg body weight of CCl_4 (20% in corn oil) twice per week for eight weeks. Group II served as the CCl_4 control and was orally administered distilled water daily. Group III served as the positive control and was orally administered silymarin (200 mg/kg) daily for 8 weeks. Silymarin is a group of flavones extracted from *Silybum marianum* L. and is a strong antioxidant. Many studies showed that silymarin was an effective agent for CCl_4 induced liver injury and hepatic fibrosis [12–15]. Groups IV and V were orally administered fraxetin powder dissolved in distilled water at doses of 20 and 50 mg/kg, respectively, daily for 8 weeks.

2.4. Serum biochemical analysis

The rats were anesthetized with urethane (1.2 g/kg, intraperitoneally (i.p.)), the blood samples from abdominal aorta were drawn into heparinized injectors, and centrifuged at 3000 rpm at 4 °C for 15 min. Serum ALT, AST and TBIL levels were measured by the first Affiliated Hospital, Wenzhou Medical College (Wenzhou, China). After finishing with the blood collection, the experimental animals were sacrificed, liver samples were dissected and washed with ice-cold saline, then they were immediately stored at −80°Cfor further analysis. The largest right lobe of each liver was excised and fixed in a 10% formalin solution for histopathologic analyses.

2.5. Measurement of lipid peroxidation and protein carbonyls

The quantitative measurement of lipid peroxidation was performed by measuring the concentration of TBARS in the liver according to the method reported by Tsaiet et al. [16]. In brief, samples were mixed with a TBA reagent consisting of 0.375% TBA and 15% trichloroacetic acid in 0.25 N hydrochloric acid.The supernatant was collected, and its absorbance was measured at 535 nm with an ELISA plate reader. Oxidative damage to proteins was quantified by the carbonyl protein assay as described previously [17]. The absorbance was measured at 370 nm with an ELISA plate reader.

2.6. Measurement of SOD, catalase and GSH-Px levels

Liver homogenates were prepared in cold Tris–HCl (5 mmol/L, containing 2 mmol/L EDTA, pH 7.4) using a homogeniser. The unbroken cells and cell debris were removed by centrifugation at 10,000 g for 10 min at 4°C. The supernatant was used immediately for the SOD, catalase and GSH-Px assays according to the protocols of commercially available kits (Jiancheng Bioengineering Institute, Nanjing, China).

Table 1
Effect of fraxetin on serum concentrations of ALT, AST and TBIL.

Groups	ALT (U/L)	AST (U/L)	TBIL (mmol/L)
Normal	47.3 ± 15.2	66.2 ± 16.2	5.5 ± 2.1
CCl_4 control	450.6 ± 80.4#	470.8 ± 75.9#	27.4 ± 7.2#
Silymarin 200 mg/kg + CCl_4	152.4 ± 27.3*	193.2 ± 55.7*	8.56 ± 3.7*
Fraxetin 20 mg/kg + CCl_4	225.7 ± 32.6*	246.3 ± 52.2*	12.5 ± 6.2*
Fraxetin 50 mg/kg + CCl_4	176.4 ± 47.8*	185.8 ± 32.9*	7.82 ± 3.2*

Rats were seven in each group. Each value represents the mean ± SD. Significance was determined by one-way ANOVA.
# $P < 0.05$ as compared with Igroup.
* $P < 0.05$ as compared with II group.

2.7. Measurement of hydroxyproline levels

The hydroxyproline levels in the livers were determined by a modified version of the previous method [18]. The liver samples were weighed and completely hydrolysed in 6 M HCl. After hydrolysis, the samples were derivatized using a chloramine T solution and Ehrlich's

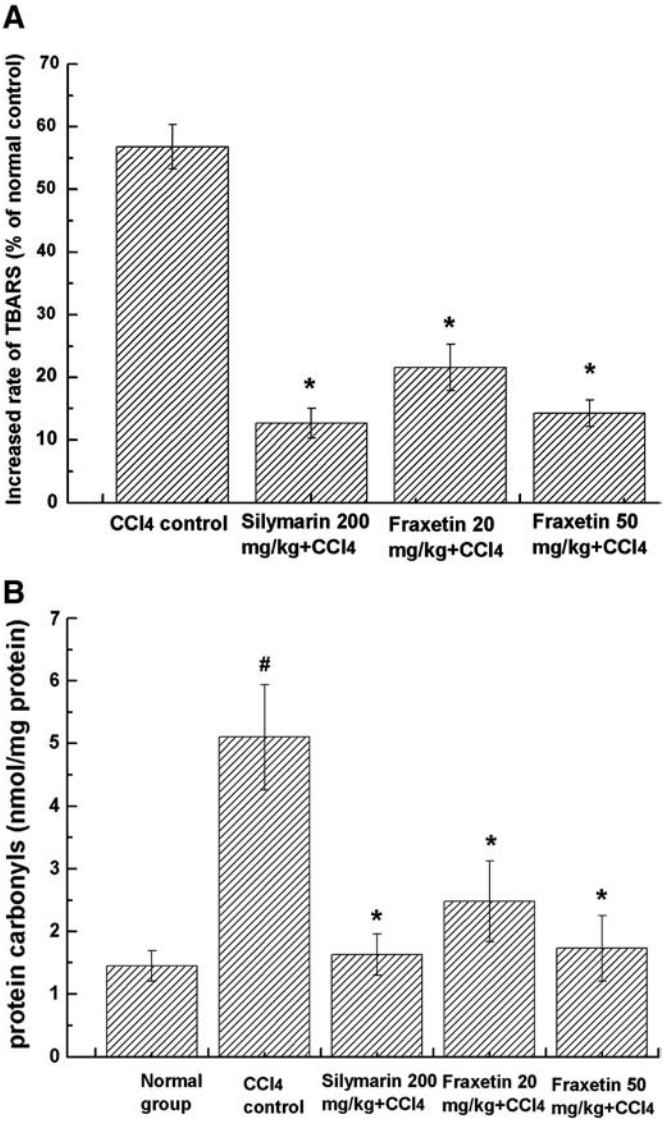

Fig. 1. Effects of fraxetin on liver TBARS (A) and protein carbonyls (B) in CCl_4 intoxicated rats. Values are the mean ± SD for 7 rats; #$P < 0.05$ as compared with normal group; *$P < 0.05$ as compared with CCl_4 treated control group.