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The protective effects of the combination of sodium ferulate and oxymatrine on ethanol-induced liver damage in mice

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ABSTRACT

The aim of this study was to investigate the effects of the combination of SF and OMT on ethanol-induced liver damage in mice. The animal liver wet/dry weight (W/D) ratio and liver tissue histopathology, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), malondialdehyde (MDA), superoxidase dismutase (SOD), C-reactive protein (CRP), interleukin-6 (IL-6), and nuclear factor κB (NF-κB) levels were measured. The data indicated that the levels of ALT, AST, TG, CRP, IL-6, NF-κB and MDA significantly decreased and that SOD activity improved after treatment with the combination of SF and OMT; the same effects were not observed with the same dose of SF or OMT when used alone. These results indicated that the combination of SF and OMT had a protective effect on ethanol-induced liver damage in mice and that antioxidation and anti-inflammatory effects might be involved in this protective mechanism.

1. Introduction

Ethanol abuse and alcoholism are serious current health problems in many areas of the world. Ethanol abuse affects many organ systems and, most notably, causes liver damage. The generation of reactive oxygen species (ROS), which increases in alcoholism, plays a major role in triggering the inflammatory response and is a central etiological factor in all types of acute and chronic liver damage pathologies (Zima and Kalousova, 2005). Currently, antioxidant drugs, such as vitamin E, vitamin C, and glutathione (GSH), are generally used in the treatment of ethanol-induced liver damage (Liu et al., 2010; Farias et al., 2012). Our previous studies found that the combination of sodium ferulate (SF) and oxymatrine (OMT) had synergetic anti-inflammatory and antioxidant effects (Yuan et al., 2011, 2012). Thus, we hypothesized that the combination of sodium ferulate (SF) and oxymatrine (OMT) might alleviate ethanol-induced liver damage and hoped to develop a potential effective drug for the treatment of ethanol-induced liver damage.

Abbreviations: SF, sodium ferulate; OMT, oxymatrine; W/D, wet/dry weight; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; MDA, malondialdehyde; SOD, superoxidase dismutase; CRP, C-reactive protein; IL-6, interleukin-6; NF-κB, nuclear factor κB; GSH, glutathione.

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The intragastric ethanol administration model has been extensively used to study the pathogenesis and intervention of ethanol-induced liver damage (Zhao et al., 2008). Using this model, the effects of the combination of SF and OMT on anti-inflammatory and antioxidant were evaluated, such as the liver wet/dry weight (W/D) ratio, liver tissue histopathology and the levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which represented the degree of the liver damage; the superoxidase dismutase (SOD) activity and malondialdehyde (MDA) level, which reflected the oxidative tissue injury and the antioxidant effect of the drug; the C-reactive protein (CRP), interleukin-6 (IL-6) and nuclear factor κB (NF-κB) levels, which could reflect the anti-inflammatory efficacy of the drugs.

2. Materials and methods

2.1. Materials

SF (molecular formula, $C_{10}H_9NaO_4{\cdot}2H_2O$; molecular weight, 252.20; CAS, 24276-84-4; HPLC purity > 99%), OMT (molecular formula, $C_{15}H_{24}N_2O_2{\cdot}H_2O$; molecular weight, 282.38; CAS, 16837-52-8; HPLC purity > 98%) were provided by Beijing SL Pharmaceutical Co., Ltd. (Beijing, China). Ethanol was produced by Yantai Sanwa Chemical Reagent Co., Ltd. (AR, Yantai, Shandong, China).

2.2. Animals

Shandong Luye Pharmaceutical Co., Ltd. supplied Swiss male mice (18–22 g) (Quality Certificate Number: Lu 20090013). The animals were kept under standard conditions (temperature, 23 ± 2 °C; humidity, 55 ± 5%; 12 h light/dark cycle) and acclimatized to the laboratory environment for 3–7 days before the experiments. Within 12 h before the experiment, only water was supplied. All experimental procedures that were conducted in this study were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by the Experimental Animal Management Center of Yantai University.

2.3. Ethanol-induced liver damage model

The ethanol-induced liver damage mouse model was duplicated following the previously described protocol (Zhao et al., 2008). The animals were randomly divided into eight groups: (1) the control group (saline), (2) the ethanol group (saline), (3) the positive control group for GSH (120 mg/kg/day), (4) the SF alone group (6.2 mg/kg/day), (5) the OMT alone group (13.8 mg/kg/day), (6) the SF + OMT low dose group (3.1 + 6.9 mg/kg/day), (7) the SF + OMT middle dose group (6.2 + 13.8 mg/kg/day), and (8) the SF + OMT high dose group (12.3 + 27.7 mg/kg/day). The optimal ratio (molar ratio = 1:2) of SF and OMT was obtained by pharmacological and pharmaceutical tests. With a 1:2 molar ratio of SF and OMT, the best pharmacological activity was demonstrated in vivo and in vitro, which would be reported in another paper. The corresponding drug or saline was intragastrically administered to the animals. One hour later, 6 g/kg ethanol was intragastrically administered to the animals, except for the control group (the animals in control group were given saline). Then, food and water were regularly provided. Additional drug or saline administrations were given at approximately 8 h and 23 h following ethanol administration.

2.4. Preparation of the serum and the tissue homogenate

Approximately one hour after the last drug or saline administration (24 h after the ethanol administration), blood sample was collected from each animal's eyeball veniplex after being anesthetized with diethyl ether. The blood samples were centrifuged at 2500 rpm for 10 min. The serum was separated and stored at −80 °C for further biochemical analysis. Then, the animals were killed humanely, and the livers were excised. The livers of mice in each group were homogenized using a Vertishear tissue homogenizer (Virtis, Gardiner, NY, USA) in phosphate buffer on ice for the 10% liver homogenate, and the homogenate was stored at −80 °C for further biochemical analysis.

2.5. W/D ratio of the liver

After the animals were killed and the livers were excised, the liver ($n = 10$) was weighed (wet weight), then dried in an oven at 70 °C for 48 h and re-weighed (dry weight). The W/D ratio was calculated using the following equation: *W/D ratio = wet weight/dry weight*

2.6. The determination of ALT, AST and TG levels

The levels of ALT, AST and TG were measured by routine laboratory methods using a Toshiba Automatic Analyzer (Toshiba, Inc., Japan).

2.7. The determination of SOD activity and MDA content

SOD activity and MDA content were assayed using SOD and MDA test kits, which were produced by the Jiancheng Bioengineering Institute (Nanjing, China). The assayed method was reported previously (Ohkawa et al., 1979; Elstner and Heupel, 1976). For SOD determination, hydroxylamines of superoxide radical anions are oxidized to form nitrite and appear violet with a color reagent. When the sample included SOD, specific inhibitory effects on superoxide radical anions inhibited the formation of nitrite, and the change in absorbance was recorded at 550 nm. The SOD activity was expressed by units, one unit was defined as a 50% inhibition of nitrite formation. For MDA determination, MDA, which is a product of the lipid peroxidation of cells, condenses with thiobarbituric acid (TBA) to form a product with a maximum absorption at 532 nm. The MDA content in the samples was determined by comparing the O.D. of the samples to the standard substance.

2.8. The determination of CRP, IL-6 and NF-κB levels

The levels of CRP, IL-6 and NF-κB in serum and in liver homogenates were measured using the enzyme-linked