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Hepatoprotective and anti-hepatocarcinogenic effects of glycyrrhizin and matrine

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ABSTRACT

Matrine (Mat), a component extracted from *Sophora flavescens* Ait, has a wide spectrum of pharmacological effects. Glycyrrhizin (Gly), a major active constituent of licorice (*Glycyrrhiza glabra*) root, has various pharmacological effects. Gly and Mat are ancillary drugs used clinically in China for protection of liver function and treatment of tumors. However, habitual administration of Gly may cause adverse effects marked by the development of pseudohypercorticosteroidism. This work was designed to see whether combination use of Gly and Mat could offer better liver protective and anti-hepatocarcinogenic effects than Gly or Mat alone, and whether it could reduce the adverse effects of Gly alone by acetaminophen-induced hepatotoxicity, diethylnitrosamine-induced hepatocarcinogenesis, induction of immunosuppression, albumen-induced swelling of rat hind paws. The results showed that compared with Gly or Mat alone, Gly + Mat reduced the mortality of acetaminophen overdosed mice more effectively, attenuate acetaminophen-induced hepatotoxicity, and reduced the number and area of γ-GT positive foci, thus protecting liver function and preventing HCC from occurring. In addition, Gly + Mat had a protective effect on immunosuppression, a strong non-specific anti-inflammatory effect, and an effect of reducing the incidence of sodium and water retention.

1. Introduction

Acute and chronic liver diseases are common and frequent occurrences in China, whose pathology and pathogenesis are complex. Some patients may progress to liver cirrhosis or hepatocellular carcinoma (HCC) with poor prognosis. Current medical treatments for these liver diseases are often ineffective, and therefore efforts are being made to seek new effective medications [1]. Developing pharmacologically effective agents from natural products has become a new trend by virtue of their little toxicity or few side effects.

Matrine (Mat) (molecular formula: $C_{15}H_{24}N_2O$), a component extracted from a traditional Chinese herb, *Sophora flavescens* Ait, is widely used in the treatment of viral hepatitis, chronic liver diseases, cardiac arrhythmia and skin inflammations in China [8,9] due to its wide spectrum of pharmacological actions including anti-inflammatory [2], immunoinhibitory [3], anti-fibrotic [4], anti-arrhythmic [5], anti-tumor [6,7] and diuretic activities without causing significant toxicity or side effects [10].

Glycyrrhizin (Gly) (molecular formula: $C_{42}H_{62}O_{16}$), a triterpene glycoside and a conjugative compound of enoxolone and glucuronic acid as an active component of licorice (*Glycyrrhiza glabra*) root, has a variety of pharmacological actions including anti-inflammatory, anti-viral, antioxidative, anti-liver cancer, immunomodulatory, hepatoprotective and cardioprotective activities [11–15]. It has been used for more than 20 years in the treatment and prevention of hepatitis, chronic bronchitis, gastritis, tumor growth and immunological disorders. However, there is evidence from numerous clinical case reports and trials that conventional administration of Gly may cause pseudohypercorticosteroidism, such as sodium and water retention, hypertension and hypokalaemia [11–13,16].

Gly and Mat are ancillary drugs used clinically in China for protection of liver function and treatment of tumors. Whether combination use of Gly and Mat could produce a better clinical effect is worth of investigation. This study was designed to compare the efficacy of Gly or Mat alone and combination use of both Gly and Mat with respect to hepatoprotective, anti-hepatocarcinogenic, immunomodulatory and anti-inflammatory activities, and see whether concomitant use of the two drugs could reduce the side effects of sodium and water retention and hypokalaemia as seen in cases of using Gly alone, and potentiate the hepatoprotective and anti-hepatocarcinogenic effects of the two drugs.

Abbreviations: Mat, matrine; Gly, glycyrrhizin; GM, mixture of Gly and Mat; PCM, acetaminophen (N-acety1-p-aminophenol, paracetamol); DENA, diethylnitrosamine; 2-AAF, 2-acetylaminofluorene; CY, cyclophosphamide; γ-GT, γ-glutamyltranspeptidase; GSH, glutathione.

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2. Materials and methods

2.1. Chemicals and reagents

Chemicals and reagents included acetaminophen, diethylnitrosamine (DEN), 2-acetylaminofluorene (2-AAF), cyclophosphamide (CY) and cortisone acetate (Jiu Zhou Pharmaceutical Co. Ltd., Shanghai, China; Sigma Chemical Co., St. Louis, US); 0.1% glycyrrhizin (Lianyungang Chia Tai Tianqing Pharmaceuticals, batch number 980414); and 0.1% Mat (Shanghai No. 1 Biochem. & Pharma., batch number 9804215).

2.2. Animals and experimental design

Sexually mature Sprague–Dawley (SD) rats, Wister rats, ICR mice were obtained from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China), housed under the conditions in a temperature and light-controlled room (23–25 °C and 14 h light:10 h dark), and fed a pellet food and water ad libitum. The animal care and permission were obtained from the Committee on Ethics of Biomedicine Research of the Second Military Medical University (Shanghai, China). The animals were equally randomized to three experimental groups and 1 or 2 control groups, each group consisting of 10–15 mice/rats and allowed 1 week to adapt to their environment before treatment. Three experimental groups were set up as follows: Gly group, using 0.1% 25 mg kg^{-1} glycyrrhizin intragastrically (i.g.); Mat group, using 0.1% 25 mg kg^{-1} Mat, i.g.; and Gly + Mat group, using a mixture of 1 g L^{-1} Gly and 1 g L^{-1} Mat 0.5 mL/20 g mice weight, i.g. The control group used the equivalent amount of water for i.g.

2.3. Acetaminophen-induced hepatotoxicity and treatment protocols

Sixty ICR mice were equally randomized to four groups. According to the experimental design, each group was administered with corresponding drugs once daily for 7 consecutive days. By the end of drug administration, the animals were injected intraperitoneally (i.p.) with 500 mg kg^{-1} 2% acetaminophen to observe 48 h mortality.

2.4. Acetaminophen-induced glutathione depletion

Fifty ICR mice of equal sexes were equally randomized to five groups. Corresponding drugs were administered according to the experimental design once daily for 7 consecutive days. Then 200 mg kg^{-1} 0.8% acetaminophen was administered i.p. by the end of drug administration. The normal control group was treated only with the equivalent amount of water i.p. The animals were killed by decapitation and the livers were dissected. Total acetaminophen-induced glutathione (GSH) was measured in liver homogenates using a total glutathione quantification kit (Dojindo Laboratories, Japan).

2.5. Diethylnitrosamine-induced hepatocarcinogenesis and treatment protocols

Forty Wister rats were equally randomized to four groups, and administered with the corresponding drugs according to the experimental design once daily for 7 consecutive days. Meanwhile, DEN was administered at 100 mg kg^{-1} i.p. daily for 2 weeks. The mice were then fed with 0.015% 2-acetylaminofluorene (2-AAF) for another 2 weeks. All groups were treated with DEN and 2-AAF to induce preneoplastic lesions of liver cancer. The left and middle lobes of liver were surgically resected on 7 days after feeding of the 2-AAF-containing food. Rats were killed by decapitation at day 3 after operation, and then the right lobes of liver were resected and fixed with ice-cold acetone/alcohol (1:1, v/v). Liver tissue specimens were paraffin-embedded for 24 h at 52–54 °C, and then stained with γ-glutamyltranspeptidase (γ-GT) according to a modified Rutenberg method. The number (foci/cm^2) and area (mm^2/cm^2) of γ-GT positive foci per square centimeter of the liver area and the average area of each focus (m^2/foci) were calculated with pathology-image analysis software.

2.6. Induction of immunosuppression and treatment protocols

Fifty ICR mice were equally randomized to five groups. Each group was administered with the corresponding drugs according to the experimental design for 13 consecutive days, during which 25 mg kg^{-1} 0.125% cyclophosphamide was administered i.p. for 7 consecutive days after the mice were treated for 4 days as above. Animals in the normal control group were injected with the equivalent amount of water for injection. Blood was drawn from the orbital cavity of the mice 24 h after the last drug administration. T lymphocyte subsets ($CD4^+T$ and $CD8^+T$) from peripheral blood were measured by flow cytometric assay. The ratio of $CD4^+T$ and $CD8^+T$ and leukocyte number were calculated.

2.7. Albumen-induced swelling of rat hind paws

Fifty SD rats were equally randomized to five groups: three experimental groups, a positive control group and a negative control group. Each group was administered with the corresponding drugs according to the experimental design once daily for 7 consecutive days. The positive control group was administered with 50 mg kg^{-1} cortisone acetate i.p., and the negative control group with the equivalent amount of water for injection. Fresh albumen 0.05 mL/claw was injected subcutaneously to the left hind claw 1 h after pretreatment, the size of which was measured at 0.5, 1, 2, 3, 4 and 5 h after albumen injection.

2.8. Determination of urine output and accumulative excretion of K^+, Na^+, and Cl^-

Forty ICR mice were equally randomized to four groups. Each group was administered with the medications according to experimental design once daily for 7 consecutive days. The mice were housed in metabolic cages 7 days after treatment. Urine was collected for 9 h to determine the output and levels of K^+, Na^+ and Cl^-.

2.9. Statistical analysis

Data were expressed as mean ± SD. All continuous variables were tested for normality by Kolmogorov–Smirnov test. Statistical comparison between groups was performed using the non-parametric Mann–Whitney-*U*, χ^2 or Dunnett's *t*-test. The comparison between two groups was performed using the Student's *t*-test. SAS (SAS Institute, Cary, NC) software package was used in the analysis. A P value ≤ 0.05 was accepted as the level of significance.

3. Results

3.1. Effects on acetaminophen-induced hepatotoxicity in mice

The results showed that combination use of Gly and Mat significantly attenuated the development of acetaminophen-induced hepatotoxicity in mice ($P < 0.05$) compared with the control group, and also increased 48 h-mortality by 20% and 26.7% compared with Gly alone and Mat alone, respectively. Gly + Mat had a bet-