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Acute and sub-chronic toxicity studies of honokiol microemulsion

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ABSTRACT

The purpose of this study was to investigate the acute and sub-chronic toxicity of honokiol microemulsion. In the acute toxicity tests, the mice were intravenously injected graded doses of honokiol microemulsion and were observed for toxic symptoms and mortality daily for 14 days. In the sub-chronic toxicity study, rats were injected honokiol microemulsion at doses of 100, 500, 2500 μg/kg body weight (BW) for 30 days. After 30 days treatment and 14 days recovery, the rats were sacrificed for hematological, biochemical and histological examination. In the acute toxicity tests, the estimated median lethal dosage (LD_{50}) was 50.5 mg/kg body weight in mice. In the sub-chronic toxicity tests, the non-toxic reaction dose was 500 μg/kg body weight. In each treatment group, degeneration or/and necrosis in vascular endothelial cells and structure change of vessel wall can be observed in the injection site (cauda vein) of a few animals while there were no changes in the vessels of other organs. The overall findings of this study indicate that the honokiol microemulsion is non-toxic up to 500 μg/kg body weight, and it has irritation to the vascular of the injection site which should be paid attention to in clinical medication.

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1. Introduction

Honokiol is a small-molecule natural component isolated from the genus *Magnolia* with two phenolic groups that confer antioxidant properties (Fig. 1). Recently, honokiol has been found to have antimicrobial (Kim et al., 2010), anti-inflammatory (Chen et al., 2014), antithrombotic (Hu et al., 2005), antitumorigenic (Bai et al., 2003; Fried and Arbiser, 2009; Ishikawa et al., 2012) and neuroprotective properties (Fukuyama et al., 2002; Hu et al., 2013; Harada et al., 2012; Zhang et al., 2013) in preclinical models. Honokiol is liposoluble and can readily cross the blood brain barrier to exert its neuroprotective effects through a wide range of mechanisms. However, its poor water solubility has caused some administration problems. In order to solve the problem of solubility and to study the protective effects on central nerve system, honokiol microemulsion has been prepared and its influence on global ischemia in mice has been investigated. The results showed that honokiol can significantly increase the breath time of mice and decrease lactic acid contents and augment ATP level in brain homogenate in this global ischemia model. The mechanism of its effect may be correlated with its alleviating ischemia status, inhibiting energy consumption, reducing MPTP opening and inhibiting PARP-1 over action, thus protects neural cells (Yang et al., 2012).

However, the information regarding the toxicity of honokiol microemulsion is very limited. This study was designed to evaluate the acute and sub-chronic toxicity of honokiol microemulsion, with the purpose of obtaining information on the safety of honokiol microemulsion to provide guidance for clinical applications.

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; APTT, activated partial thromboplastin time; BW, body weight; BUN, urea nitrogen; CK, creatine kinase; CRE, creatinine; FIB, fibrinogen; GLU, glucose; HCT, hematocrit; HGB, hemoglobin; IC_{50}, half maximal inhibitory concentration; ICR, imprinting control region; LD_{50}, median lethal dosage; LDH, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NOAEL, no observed adverse effect level; PARP, poly ADP-ribose polymerase; PLT, platelets; PT, prothrombin time; RBC, red blood cells; SD, Sprague Dawley; TBIL, total bilirubin; TCHO, total cholesterol; TG, triglyceride; TT, thrombin time; TP, total protein; UA, uric acid; WBC, white blood cells.

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2. Materials and methods

2.1. Test material

Honokiol microemulsion is a slight yellow oily liquid with the content of 10 mg/ml developed by Pharmaceutical Sciences School of Peking University (Beijing, China). During the study, the test article was stored in the dark with a temperature of 2–8 °C and dissolved in a 0.9% saline solution (Shanxi YunPeng Pharmaceutical Co., Ltd., China) freshly before use.

2.2. Animals

Fifty 4–5 week-old ICR mice of both sexes and one hundred and twenty 5–7 week-old SD rats of both sexes were obtained from the Laboratory Animal Center of Academy of Military Medical Sciences of China. Upon arrival, all animals were examined for health condition to confirm the suitability for study and the mice were allowed to acclimate to the laboratory environment for 5 days and the rats for 7 days. The animals were housed by sex in groups of five per cage in an environmental-controlled barrier-sustained animal room, and supplied with standard commercial diet and drinking water *ad libitum*. With the exception of minor variations, all animal rooms were monitored and maintained under a 12 h light–dark cycle, with temperature ranging from 20 to 25 °C and relative humidity varied between 40% and 70%. This study was approved by the Institutional Animal Ethics Committee of New Drug Safety Evaluation Center in the Institute of Materia Medica before start.

2.3. Acute toxicity

A total of fifty mice were assigned randomly to five groups of five males and five females each. The honokiol microemulsion was injected through caudal vein at grade doses of 41, 51.2, 64, 80, 100 mg/kg body weight. The general behavior of mice and signs of toxicity were observed continuously for 3 h after injection. The mice were further observed once a day up to 14 days for behavioral changes and signs of toxicity and/or death. The body weights were monitored on day 0, 3, 7 and 14, and their food consumption was monitored on days 0, 3 and 12.

2.4. Sub-chronic toxicity

SD rats of both sexes were assigned randomly to four groups (three treatment group and one vehicle group) of 15 males and 15 females each. The rats in the vehicle group were injected 0.9% saline through caudal vein, and the rats in treatment groups were injected 100, 500 or 2500 μg/kg body weight of honokiol microemulsion, respectively, once a day for 30 days. Two thirds of the animals, half males and half females, were sacrificed 24 h after the final administration on day 31 (D31), and the rest third were sacrificed at the end of a 2-weeks recovery period on D45 for blood collection and histopathologic examination to observe the recovery and delayed toxicity that might occur. The animals were observed closely for any behavioral changes every day. The body weights of animals and food consumption were monitored weekly through the study period.

2.5. Measurement of biochemical and hematological parameters in blood

Hematology, serum biochemistry, and coagulation evaluations were performed for 10 animals/sex/group on D31 (termination of treatment) and for 5 animals/sex/group on D45 (termination of recovery). All rats were fasted overnight for more than 12 h prior to blood collection. Blood samples were collected through abdominal aorta puncture for hematology and serum biochemistry after the rats were anesthetized with pentobarbital sodium by intraperitoneal injection.

2.5.1. Hematological and coagulation parameters

The blood samples collected in the $EDTAK_2$-coated vials were analyzed using an auto-hematology analyzer (MEK-6318K, Nihon Kohden) for white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and platelets (PLT).

Blood sample were collected into sodium citrate-coated vials, plasma was separated for coagulation parameters, such as prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (FIB) and thrombin time (TT), using a semi-automated coagulation analyzer (STA-4, Stago Co., Ltd.).

2.5.2. Serum biochemistry

The blood biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), urea nitrogen (BUN), creatinine (CRE), total cholesterol (TCHO), glucose (GLU), total bilirubin (TBIL), triglyceride (TG), creatine kinase (CK), lactate dehydrogenase (LDH) and uric acid (UA) were determined using an automatic biochemistry meter (SELECRTA-E, Vital Scientific). K^+, Na^+, Cl^- and Ca^{2+} were determined using the ion-selective electrode method with an AC980 electrolyte analysis instrument (Audicom Medical Instruments Co., Ltd.).

2.6. Organ weight and histopathology

After blood collection, the animals were sacrificed and the organs, including brain, spinal cord, pituitary, sternum, thymus, thyroid, parathyroid, esophagus, salivary glands, stomach, small/large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, testes, epididymis, uterus, ovaries, female mammary gland, prostate, urinary bladder, lymph nodes, sciatic nerve and caudal vein (injection site) were isolated for histological examination. We also determined the absolute and relative organ weights (based on terminal body weights) for the brain, heart, liver, spleen, kidneys, lungs. The relative organ weights were calculated as follows:

$$\text{Relative organ weight} = \text{Absolute organ weight (g)}/\text{Body weight (g)} \times 100\%. \tag{1}$$

For the histological examination, all organs and tissues were fixed in 10% formalin, dehydrated with varying grades of alcohol, embedded in paraffin, cut into standard thick sections and stained with hematoxylin–eosin dye for microscopic observation.

2.7. Statistical analysis

All data are expressed as the mean ± standard error of the mean (S.E.M) and comparisons among different groups were performed by analysis of variance using an ANOVA test and DAS 1.0 statistical software. The LD_{50} value was determined according to the Bliss method (Bliss, 1938).

3. Results

3.1. Acute toxicity

The mortality as well as the acute toxicity increased progressively as the dose increased from 41 to 100 mg/kg (Table 1). All

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