Regulatory Toxicology and Pharmacology 95 (2018) 362–370

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Acute and subchronic toxicities in dogs and genotoxicity of honokiol microemulsion

Xiujun Qin[a,b], Jingjing Yin[a,b], Wei Zhang[a,b], Jianguo Li[a,b], Jianhua Wen[a,b,*], Shizhong Chen[c,**]

[a] Department of Radiological and Environmental Medicine, China Institute for Radiation Protection, Taiyuan 030006, China
[b] Shanxi Key Laboratory of Drug Toxicology and Drug for Radiation Injury, Taiyuan 030006, China
[c] School of Pharmaceutical Sciences, Peking University, Beijing 100191, China

ARTICLE INFO

Keywords:
Honokiol microemulsion
Acute toxicity
Subchronic toxicity
Genotoxicity
Beagle dog

ABSTRACT

This article aims to conduct toxicity test research on honokiol microemulsion(HM) to provide reference frame for the safe dose design as well as the toxic and adverse reaction monitoring in clinic. High performance liquid chromatography (HPLC) method was adopted to determine the concentration, stability and uniformity of HM and the results indicated that the test sample was conformed to the toxicity test requirements. In the acute toxicity test, six intravenous drip dosages, namely, 100.0, 66.7, 44.4, 19.8, 8.8, and 3.9 mg/kg were set, with one beagle dog in each dosage, respectively. In addition, the results also demonstrated that the approximate lethal dose range of HM was 66.7–100.0 mg/kg. In the subchronic toxicity test, beagle dogs were intravenously dripped with HM at doses of 1.25, 0.25 and 0.05 mg/kg for 30 days. During the test period, signs of gross toxicity, behavioral changes, body weight, rectal temperature, food consumption, ophthalmoscopy, electrocardiography, urinalysis, blood biochemistry, coagulation, hematology, organ weights and histopathology were examined. Under the present study conditions, the no-observed-adverse-effect level for HM was estimated to be 0.25 mg/kg. According to the results of bacterial reverse mutation, chromosomal aberration and micronucleus assays, HM exhibited no notable genotoxicity both in vivo and in vitro.

1. Introduction

Honokiol (CAS number: 35354-74-6), a bioactive compound, is a main biphenyl neolignan extracted from traditional Chinese medicinal herb Hou pu, cortex of Magnolia officinalis (Magnoliaceae). It is insoluble in water, and its chemical structure is shown in Fig. 1 with a variety of pharmacological effects such as antioxidation (Lo et al., 1994; Chiu et al., 1997; Liou et al., 2003), anti-platelet aggregation (Teng et al., 1988), antianxiety (Kuribara et al., 2000), antibacterial (Ho et al., 2001), anti-inflammatory (Liou et al., 2003), antiarrhythmic (Hong et al., 1996), antitumor (Hibasami et al., 1998) and neurotrophic effects (Zhai et al., 2003).

Thrombosis is a major cause of cardiovascular and cerebrovascular diseases. Honokiol exerts protective effect on cerebral ischemia (Liou et al., 2003; Hu et al., 2006) and the mechanism of action may be related to neurotrophy (Fukuyama et al., 2002), anti-oxidation (Liou et al., 2003), and anti-inflammatory (Liou et al., 2003). Recent research reported that, honokiol can inhibit platelet aggregation and arterial thrombosis through stimulating PGI_2 generation in endothelial cell (Hu et al., 2005) and honokiol also can protect brain against global brain ischemia through inhibiting MPTP opening and PARP-1 activity (Yang et al., 2012). Therefore, honokiol microemulsion (HM) is proposed for clinically treating ischemic stroke.

HM was developed by Pharmaceutical Sciences School of Peking

Abbreviations: 2-AF, 2-aminofluorene; 4-NQO, 4-Nitroquinoline-N-oxide; A/G, albumin/globulin; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; b, chromatid break; BA, basophile granulocte; BIL, bilirubin; BLD, occult blood; BUN, urea nitrogen; CHL, Chinese hamster lung fibroblast; CK, creatine kinase; CP, cyclophosphamide; CRE, creatinine; dic, dicentric chromosome; EO, eosinophile granulocyte; FIB, fibrinogen; GGT, gamma-glutamyl transferase; GLU, glucose; HCT, hematocrit; HGB, hemoglobin; HM, honokiol microemulsion; HPLC, high performance liquid chromatography; i, chromatid exchange; KET, ketone; LDH, lactate dehydrogenase; LEU, leucocyte; LY, lymphocyte; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MMC, mitomycin C; MO, monocyte; MPV, mean platelet volume; NaN3, sodium azide; NCE, normochromatic erythrocytes; NE, neutrophilic granulocyte; NIT, nitrite; p, polyploidy; PCE, polychromatic erythrocytes; PCT, plateletocrit; PDW, platelet distribution width; PLT, platelet; PRO, protein; PT, prothrombin time; r, ring chromosome; RBC, red blood cell; RDW, red cell distribution width; Reti, reticulocyte; RSD, relative standard deviation; SG, specific gravity; TBIL, total bilirubin; TCHO, total cholesterol; TG, triglyceride; TP, total protein; TT, thrombin time; UA, uric acid; UBG, urobilinogen; WBC, white blood cell

* Corresponding author. Department of Radiological and Environmental Medicine, China Institute for Radiation Protection, No. 102 Xuefu Street, Taiyuan, China.
** Corresponding author. School of Pharmaceutical Sciences, Peking University, No. 38 Xueyuan Road, Beijing, China.
E-mail addresses: jianhua570621@163.com (J. Wen), chenshizhong66@163.com (S. Chen).

https://doi.org/10.1016/j.yrtph.2018.04.010
Received 3 September 2016; Received in revised form 8 April 2018; Accepted 11 April 2018
Available online 12 April 2018
0273-2300/

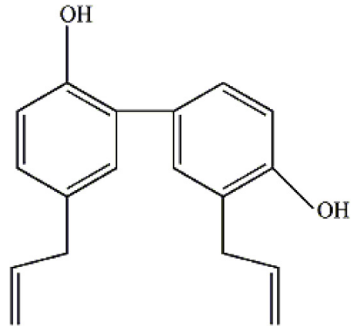

Fig. 1. Chemical structure of Honokiol.

University, and the in vivo pharmacokinetic study of honokiol discovered that it was not suitable for single dose form of intravenous infusion (Chen et al., 2004). As a result, it was formulated into the microemulsion dose form through pharmaceutical method, which could not only increase the solubility, but also could extend the release duration. We had reported the studies of acute and subchronic toxicity of HM in rodent (Zhang et al., 2015), and the present studies were conducted as part of an investigation to examine the safety of HM, thus providing data for its clinical research.

2. Materials and methods

2.1. Honokiol microemulsion

HM (purity > 99%), a slight yellow oily liquid with the content of 10 mg/ml, was synthesized from Pharmaceutical Sciences School of Peking University (Beijing, China). Before use, the HM dissolved in a 0.9% saline solution freshly.

2.2. Concentration, stability and uniformity analysis of HM

The high performance liquid chromatography (HPLC) with ultraviolet detection was utilized to determine the concentration, stability and uniformity of HM (Allance 2695–2996, Waters, USA). Besides, chromatographic separation was performed on a Diomonsil™ C_{18} column (250 mm × 4.6 mm) with methanol-water (80: 20, v/v) as the mobile phase. The flow rate was 1.0 ml/min, and injection volume reached 10 µl. The UV detection wavelength was 294 nm.

Honokiol purchased from National Institutes for Food and Drug Control was used as standard substance. To prepare the standard solution, methanol was added to dissolve 5.4 mg standard substance and the final solution volume was 100 ml. In addition, content was treated as the horizontal axis and the peak area was treated as the vertical axis. Then, the standard curve was drawn, and the regression equation could be obtained.

When the concentration of HM was determined, methanol was added to dissolve 0.5 ml HM and the final solution volume was 100 ml. After preparation, the stability and uniformity analysis of HM were conducted at 0 and 4 h. In the uniformity analysis, the honokiol content in the upper, middle and lower layers of HM with various concentrations were detected, respectively. For each sample, the detection was repeated for three times, and the relative standard deviation (RSD) values were calculated.

2.3. Animals

The beagle dogs were purchased from Weiguang Experimental Animal Center of Fuyang City (Fuyang, China). The ICR mice were purchased from the Laboratory Animal Center of Academy of Military Medical Sciences of China (Beijing, China). In the acute toxicity test, six beagle dogs of both sexes were 6-7-month-old weighting 8–10 kg. In the subchronic toxicity test, twenty-four beagle dogs of both sexes were 6-8-month-old weighting 6–9 kg. In the genotoxicity test, thirty-six male ICR mice were 4-5-week-old weighting 18–21 g. The animals were housed in a temperature-controlled and humidity-monitored room with a 12-h light/dark cycle. Standard commercial mouse and dog food (Beijing Keao Xieli Feed Co, Ltd.) and sterile water were provided ad libitum. The beagle dogs were acclimatized for 2 weeks and mice were 3 days before use in the experiments. This study was approved by the Institutional Animal Ethics Committee of China Institute for Radiation Protection before start.

2.4. Acute toxicity study

Six dogs with random gender were allocated into six dosages of one animal each. The concentrations of HM were 10.0, 6.67, 4.44, 1.98, 0.88 and 0.39 mg/ml. The dogs were intravenous injected HM at doses 100.0 (female), 66.7 (male), 44.4 (male), 19.8 (female), 8.8 (female), and 3.9 (male) mg/kg of body weight. The dosing volume was 10 ml/kg, and rate of administration slower than 5 ml/min. Each animal was observed for signs of toxicity and behavioral changes continuously for 3 h after test sample administration and once daily thereafter for 14 days. Mortality checks were performed daily. Body weights, temperature, electrocardiography, blood biochemistry, and hematology were recorded before administration and on days 5 and 15. Animals were observed for their death and subjected to a gross necropsy immediately. On day 15, the survival dogs were sacrificed for macroscopic findings and the abnormal organs were isolated for histological examination.

2.5. Subchronic toxicity study

2.5.1. Experimental design

24 beagle dogs of both sexes were assigned to four groups (control and three treatment groups) of six in each group consisting of three males and three females. The concentrations of HM were 0.25, 0.05 and 0.01 mg/ml. HM of 1.25, 0.25 and 0.05 mg/kg body weight were intravenous dripped once per day for 30 days and the control group was administered with physiological saline. The dosing volume was 10 ml/kg, and rate of administration slower than 5 ml/min. Animals were observed daily for overt signs of toxicity. Throughout the study, body weights, food consumption and rectal temperature were recorded weekly. Ophthalmological examination was conducted on days 30 and 44. Electrocardiography, urinalysis, hematological, biochemical and coagulation parameters were conducted on days 15, 30 and 44. On day 31, 2 males and 2 females of each group were necropsied, and the remaining animals were continuously observed for another 2 weeks.

2.5.2. Ophthalmoscopy and electrocardiography

The eyelid, conjunctiva, cornea, iris, pupil and fundus of each animal were examined for abnormalities of the eyes. Electrocardiogram (lead II) of each animal was taken for heart rate, QRS complex, PR interval, ST interval, and QT interval by using electrocardiography (ECG-6951E, NIHON KOHDEN, Japan).

2.5.3. Urinalysis

Urine samples were tested for parameters including color, bilirubin (BIL), urobilinogen (UBG), ketone (KET), glucose (GLU), pH, nitrite (NIT), leucocyte (LEU), specific gravity (SG), protein (PRO), and occult blood (BLD). The urinalysis was performed using reagent strips that were read using a urine chemistry analyzer (CLINITEK 50, Bayer, USA).

2.5.4. Hematological, biochemical and coagulation parameters in blood

The blood samples collected in the EDTA-2K-coated vials were analyzed for white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet (PLT),