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Embryo-fetal development toxicity of honokiol microemulsion intravenously administered to pregnant rats

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ABSTRACT

The aim of this study was to evaluate the embryo-fetal development toxicity of honokiol microemulsion. The drug was intravenously injected to pregnant SD rats at dose levels of 0, 200, 600 and 2000 μg/kg/day from day 6–15 of gestation. All the pregnant animals were observed for body weights and any abnormal changes and subjected to caesarean-section on gestation day (GD) 20; all fetuses obtained from caesarean-section were assessed by external inspection, visceral and skeletal examinations. No treatment-related external alterations as well as visceral and skeletal malformations were observed in honokiol microemulsion groups. There was no significant difference in the body weight gain of the pregnant rats, average number of corpora lutea, and the gravid uterus weight in the honokiol microemulsion groups compared with the vehicle control group. However, at a dose level of 2000 μg/kg/day, there was embryo-fetal developmental toxicity observed, including a decrease in the body length and tail length of fetuses. In conclusion, the no-observed–adverse-effect level (NOAEL) of honokiol microemulsion is 600 μg/kg/day, 75 times above the therapeutic dosage and it has embryo-fetal toxicity at a dose level of 2000 μg/kg/day, which is approximately 250 times above the therapeutic dosage.

1. Introduction

Ischemic stroke is one of the leading causes of mortality and long-term disability worldwide, and remains a serious and significant global health problem. Generally, thrombolytic treatments can effectively improve chances of survival after an acute ischemic stroke, for example the use of recombinant tissue plasminogen activator (rt-PA) (Sauer et al., 2010; Yoo et al., 2013). However, a further progression of neuronal damage after ischemia-reperfusion may cause serious disability to patients not getting effective treatment. Therefore, neuroprotective therapy during ischemia-reperfusion is important to reduce the neuronal injury.

Honokiol, a major bioactive component isolated from the bark of *Magnolia officinalis* with two phenolic groups that confer antioxidant properties (Fig. 1), was used in Chinese traditional medicine for thousands of years. 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; Harada et al., 2012; Hu et al., 2013; Zhang et al., 2013) in preclinical models. Honokiol exerts its neuroprotective effects through inhibition of the immune system and oxidative stress pathways (Chen et al., 2007; Harada et al., 2012). Because of its lipid solubility, honokiol

Abbreviations: AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care; GD, gestation day; GLP, Good Laboratory Practice; ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; LOAEL, lowest-observed–adverse-effect level; MPTP, mitochondria permeability transition pore; NMDA, N-methyl-D-aspartic acid; nNOS, neuronal nitric oxide synthase; NOAEL, no-observed-adverse-effect level; PARP, poly ADP-ribose polymerase; PSD95, postsynaptic density protein 95; rt-PA, recombinant tissue plasminogen activator; SD, Sprague Dawley; SOD, reactive oxygen species.

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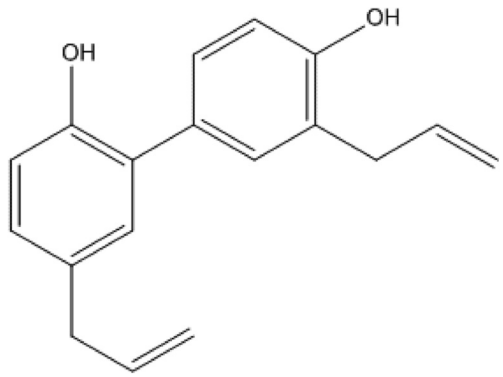

Fig. 1. Chemical structure of honokiol.

can readily cross the blood brain barrier to treat diseases in the central nervous system. Preclinical researches have revealed that it could relieve ischemia-reperfusion injury in rodent model (Liou et al., 2003a,b). However, its poor water solubility has caused some administration problems.

Honokiol microemulsion, a new dosage form of honokiol developed by School of Pharmaceutical Sciences, Peking University has good water solubility, and the investigation about its influence on global cerebral ischemia in mice showed that it can significantly increase the breath time of mice and decrease lactic acid contents and augment ATP level in brain homogenate. The mechanism of its effect may be correlated with its alleviating ischemia status, inhibiting energy consumption, reducing mitochondria permeability transition pore (MPTP) opening and inhibiting poly ADP-ribose polymerase-1 (PARP-1) over action, thus protects neural cells (Yang et al., 2012).

However, the literature pertaining to the reproductive toxicity of honokiol, as an important part of preclinical toxicology profile, is limited and incomplete. Therefore, in consideration of the potential toxic effects of honokiol microemulsion, we assessed the embryo-fetal development toxicity of honokiol microemulsion in rats. The study was performed under Good Laboratory Practice (GLP) regulations and was designed and run according to ICH guidelines (ICH, 2005) in the laboratory that passed the certification of AAALAC.

2. Materials and methods

2.1. Test materials

The test material, honokiol microemulsion, exists as a slight yellow oily liquid with the content of 10 mg/ml developed by Pharmaceutical Sciences School of Peking University (Beijing, China). Analyses of the test material demonstrated that it has good water solubility and homogeneity, and can keep stable for 2 years if stored in the dark below the temperature of 4 °C. During the study, honokiol microemulsion was stored in the dark at a temperature of 4 °C and dissolved in a 0.9% saline solution (Shanxi YunPeng pharmaceutical Co., Ltd, China) freshly before use. Saline was singly used as vehicle control, while sodium salicylate (Sinopharm chemical reagent Co., Ltd), dissolved in water, was administered intragastricly as positive control in the study (Davis et al., 1996; Warkany and Takacs, 1959).

2.2. Animals and husbandry

The Sprague Dawley (SD) rats, including 30 males and 120 females, obtained from Vital River Laboratories were 60–100 days old, healthy and nulliparous. Throughout the study, the animals were housed by sex in groups of five (except during mating) in transparent polypropylene cages with stainless steel wire lids in an environmental-controlled barrier-sustained animal room, and supplied with standard breeding diet obtained from Laboratory Animal Center of Academy of Military Medical Sciences of China (Batch No. 20120412, 20120503, 20120510; Certification No. 0002255, 0002300) 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 a temperature of 22.7 ± 0.9 °C and relative humidity of 60.5 ± 5.0% throughout this study. After one week acclimation and quarantine, males and females were used to copulate. For mating, four females were placed into the cage of each male overnight. On the following morning and every morning thereafter, the females with spermatozoa in vaginal smear and/or copulatory plugs *in situ* were considered to be at gestation day (GD) 0, and assigned to one of five dose groups by computerized random selection.

2.3. Dose selection and administration

The anticipated dose of honokiol microemulsion is 0.8–8 μg/kg through intravenous infusion in clinical use. According to the acute and sub-chronic toxicity studies of honokiol microemulsion we conducted previously, the estimated LD_{50} of the test material was 50.5 mg/kg body weight in mice and the NOAEL of honokiol microemulsion was considered to be 500 μg/kg in SD rats (Zhang et al., 2015). On the basis of these results, the dose of 200, 600 and 2000 μg/kg/day were selected as low-, mid-, and high-doses, respectively.

This study was comprised of five groups of 22–24 copulated female rats each, a vehicle control group (saline), three honokiol microemulsion groups (200, 600, 2000 μg/kg/day honokiol microemulsion), and a positive control group (250 mg/kg/day sodium salicylate). Accurate 200, 600 and 2000 μg/ml honokiol microemulsion solutions were prepared immediately before using for low-, mid-, and high-dose groups, respectively, and intravenously injected to pregnant rats from GD 6 to 15 with a dose volume of 1 ml/kg/day once daily, consistent with the route of administration in clinical use. Sodium salicylate was diluted in distilled water and administered intragastricly from GD 8 to 10 with a dose volume of 10 ml/kg/day once daily. The administration route of vehicle control group was the same as the honokiol microemulsion groups.

2.4. Body weights and clinical observation

All dams were weighed on GD 0, 3, 6, 9, 12, 15, 18 and 20. Maternal body weight gain during GD 6–20 was calculated. Animals were observed closely for signs of overt toxicity, abnormal changes in general appearance and behavior, morbidity and mortality once daily throughout the treatment period.

2.5. Caesarean-section observations

On GD 20, all females of each group were weighed, sacrificed and necropsied with observation of the ovaries and evaluation of uterine contents. The ovaries were examined and the numbers of corpora lutea were recorded. The uteruses were excised, weighed, and the numbers of implantation sites, resorption sites, live and dead fetuses were recorded respectively, as well as the weight of live fetuses and placentas. Resorption was discriminated from dead fetuses when only embryonic tissue was visible at caesarean section without morphological integrity of fetuses. Uteri from apparently non-gravid female were stained with 10% sodium sulfide to

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