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Chronic toxicity of ginsenoside Re on Sprague-Dawley rats

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ABSTRACT

Ethnopharmacological relevance: Ginseng has been widely used for hundreds of years in both China and other countries. It is well accepted that the pharmacological effects of ginseng are attributed to ginsenosides. Ginsenoside Re is one of the active ingredients in ginseng. The present study was carried out to characterize the toxicity of ginsenoside Re after repeated oral administration in Sprague-Dawley rats.

Materials and methods: Rats (60 males, 60 females) were administrated ginsenoside Re orally in 0, 38, 113, or 375 mg/kg/day doses for 26 weeks (n=15/group each sex). Clinical signs, mortality, body weights, feed consumption, urinalysis, hematology, serum biochemistry, gross findings, organ weights and histopathology were examined at the end of the test period, as well as after the 4-week recovery period.

Results: Ginsenoside Re did not induce death, adverse effects or dose-dependent changes in feed consumption, or body weight gain. Some statistically significant differences were observed in hematological and biochemical parameters, as well as in body weights of rats treated with ginsenoside Re. However, there was no abnormality of any organs noted in both gross and histopathological examinations.

Conclusions: Ginsenoside Re is well tolerated up to a 375 mg/kg/day oral dosage level and non-toxic in both male and female rats.

1. Introduction

Ginseng has been extensively used by both patients and healthy individuals in many countries to restore and enhance vital energy (Chong and Oberholzer, 1988). It is well accepted that pharmacological effects of ginseng are mostly attributed to ginsenosides, the principal components in different species of ginseng. In general, the main active ginsenosides are categorized into two groups, 20(*S*)-protopanaxatriol (PPT-type) and 20(*S*)-protopanaxadiol (PPD-type), based on the number and the position of the sugar moieties attached on the dammarane skeleton (Leung et al., 2007). It has been reported that differences in the structural conformation and presence of sugars at positions C-3 and C-6 are responsible for unique characteristics of different compounds and affected their hydrophobic properties required to functionally interact with cell membrane. For the hydrophobic properties of ginsenosides effect in the permeability of cell membrane, see Popovich and Kitts (2002). Furthermore, molecular modeling and sugar moiety position also influenced the ginsenosides activity (Chen et al., 2009; Qi et al., 2010).

Ginsenoside Re (Re) is one of the PPT-type ginsenosides. Several typical bioactivities of Re have been found to be anti-diabetic (Xie et al., 2005; Cho et al., 2006a,b; Zhang et al., 2008;Yang et al., 2010; Liu et al., 2012), antioxidant (Xie et al., 2006; Cho et al., 2006a, b), immuno-potentiating (Song et al., 2010; Son et al., 2010;Chan et al., 2011), neuro-protective (Ji et al., 2006; López et al., 2007), and angiogenic modulating activity (Leung et al., 2007; Yu et al., 2007; Li and Liu, 2008). Our previous studies indicated that Re improved learning and memory in rats by enhancing the basic synaptic transmission and promoting the magnitude of LTP of the dentate gyrus (Zhao et al., 2007).

Despite of the long history of usage and widespread research on ginseng activities, the chronic effects of ginseng are not well characterized and little information on toxicity is available (Chan and Fu, 2007). Chronic toxicity studies of gingseng extract were performed, and no toxic effects were found (Popov and Goldwag, 1973; Bittles et al., 1979; Hess et al., 1982, 1983). However, the dose level used in gingseng extract toxicity studies was considered low (Chan and Fu, 2007). Recently, our groups demonstrated the chronic-toxicity of 20(*S*)-ginsenoside Rg3 in Beagke dogs (Liu et al., 2011). In another, subchronic toxicity study, the hepatotoxicity of ginsenoside CK was also reported (Gao et al., 2011). Both ginsenoside CK and Rg3 are PPD-type ginsenosides, and there are no reports about the chronic toxicity of PPT-type ginsenoside. In order

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to know the comprehensive toxicological properities of ginsengs and ginseng products, and establish an appropriate dosage of Re in clinical test, we undertook a chronic toxicological study on SD (Sprague-Dawley) rats to characterize the toxicity of Re.

2. Materials and methods

2.1. Test article and treatment

The chemical structure of Re is depicted in Fig. 1. Re with a chemical purity of 98.5% (determined via HPLC) was obtained from the Laboratory of New Drugs Research, Institute of Frontier Medical Science, Jilin University. The fruits of *Panax quinquefolius Linn.* (Araliaceae) were collected in the Jingyu county of Jilin province, China. Fresh fruits *P. quinquefolium Linn.* were pulverized and filtrated to obtain juice, the residue was extracted with water and then filtrated to get a water soluble fraction. The water soluble fraction combined with the juice was subjected to column chromatography on highly porous polymer column D101, and was eluted with distilled water and then 95% EtOH. The EtOH fraction was enriched in Re. Then Re was purified by silica gel column chromatography and recrystallized.

The study was performed following the Good Laboratory Practice of the People's Republic of China. This nonclinical laboratory study was carried out in compliance with the Testing Guidelines for Safety Evaluation of Drugs (Notification [H] GPT2-1 issued by China Food and Drug Administration on March 2005) and the Organization for Economic Cooperation and Development under the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies. These studies were conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International, and the animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals.

2.2. Animals and management

A total of 120 Sprague-Dawley (SD) rats (60 males, 60 females) were obtained from B&K Universal Group Limited (Shanghai, China) at 5–6 weeks of age, followed by 1 week of quarantine and acclimatization. The animals were maintained at a room temperature of 22 ± 2 °C and a relative humidity of 55 ± 15% with lamp light and noise below 6 dB. The animals were housed individually in cages and supplied with feed and water *ad libitum* in cages.

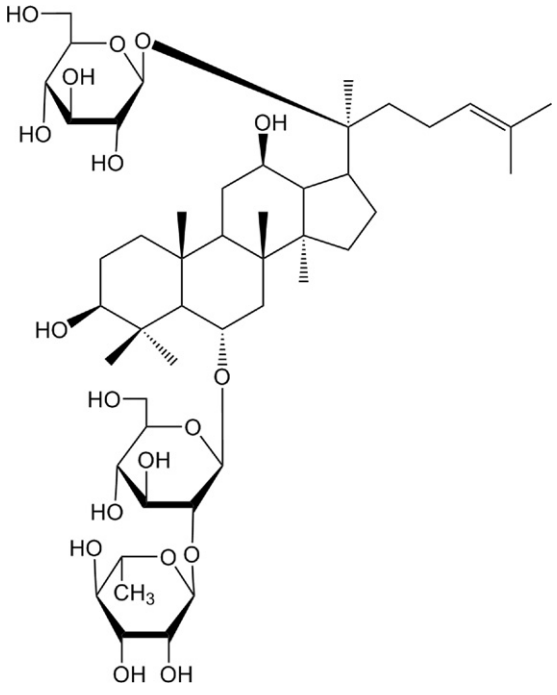

Fig. 1. Chemical structure of ginsenoside Re.

2.3. Study design and dose selection

Healthy rats of both sexes were randomly divided into one of four treatment groups (15 males and 15 females): three experimental groups (low-, mid-, high-dose group), in which animals were administrated orally Re 38, Re 113, or Re 375 mg/kg/day respectively, and one control group in which animals were orally administrated water only. Water was used as a vehicle for the Re doses. In each group, all of the rats were administrated orally daily up to 26 weeks. After 13 weeks, 4 rats per each sex in each group were sacrificed. Another 6 rats per each sex in each group were sacrificed at the end of the 26th week. The remaining rats were sacrificed after 4 weeks of recovery. The animals of the recovery groups were observed for reversibility, persistence and delayed occurrence of toxic effects.

The dosages selected in the present study were based on existing data on the effective dose, and the results of an acute toxicity study of Re on mice. In the acute toxicity study, the LD_{50} was considered to be more than 5000 mg/kg with oral administration, and the maximum tolerance dose (MTD) was considered to be more than 90 mg/kg with oral administration. In the present study, 375 mg/kg was specified as the highest dose level, with lower doses being 113 mg/kg and 38 mg/kg, and these were respectively 100, 30 and 10 times the clinical application dosage.

2.4. Clinical observation

The animals were observed daily prior to and following administration for signs of toxicity and mortality throughout the experimental period. Detailed clinical signs were assessed and recorded, including, but not limited to, changes in skin and fur, eyes and mucous membranes, manure, psyche states and behavior patterns. The body weight was measured at the initiation of treatment and once a week during the treatment period thereafter. The amounts of feed were weighed before they were supplied to each cage and their remnants were measured the next day. The differences were calculated, which were regarded as daily feed consumption (g/animal/day).

2.5. Laboratory testing parameters

Samples of blood were obtained from all animals on Day 92 and Day 183 of the study, and after recovery (one time for each animal). All rats were fasting but allowed access to water *ad libitum* for more than 12 h prior to collecting a blood sample. Obtained from the abdominal aorta, blood samples were stored in evacuated blood collection tubes. EDTA–K_2 was used as the anticoagulant for blood coagulation study. The hematological parameters, including erythrocyte counts (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets count (PLT), and leukocyte counts (WBC), were assessed using an AC-900 Automated Hematology Analyzer (JieRui Medical Reagent Co., AnShan, ShenYang, China). The coagulation parameters, including prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), and fibrinogen (FIB), were evaluated using a mechanical KC-4A coagulometer (Amelung, Lemgo, Germany). Blood smears were stained with Wright–Giemsa brilliant-cresyl-blue, and the reticulocyte count (Ret) was calculated under light microscopy.