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Sulfur fumigation reducing systemic exposure of ginsenosides and weakening immunomodulatory activity of ginseng

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ABSTRACT

Ethnopharmacological relevance: Ginseng (Ginseng Radix et Rhizoma) is used worldwide for its miracle tonic effects, especially for its immunomodulatory activities. Sulfur fumigation, a fast and convenient method to prevent pesticidal and bacterial contamination in the food industry, has been recently employed during post-harvest processing of ginseng. Our previous studies demonstrated that sulfur fumigation significantly altered the chemical profile of the bioactive ingredients in ginseng. However, the effects of sulfur fumigation on the pharmacokinetics and bioactivities of ginseng remain unknown.
Aim of the study: To examine the effects of sulfur fumigation on the pharmacokinetics and immunomodulatory activities of ginseng.
Materials and methods: For pharmacokinetic studies, male Sprague-Dawley rats exposed to single/multiple dosages of non-fumigated ginseng (NFG) and sulfur fumigated ginseng (SFG) were investigated using HPLC-MS/MS analysis. For bioactivity studies, male ICR mice were used to compare the immunomodulatory effects of NFG or SFG under both normal and cyclophosphamide (CY)-induced immunocompromised conditions using white blood cell counts, serum cytokine levels, and spleen and thymus weight indices.
Results: Sulfur fumigation significantly reduced the contents of the bioactive ginsenosides in ginseng, which resulted in drastically low systemic exposure of ginsenosides in SFG-treatment group compared to NFG-treatment group. This observation was consistent with the bioactivities obtained in NFG- and SFG-treatment groups. The bioactivity studies also demonstrated the immunomodulatory effects of NFG but not SFG in the CY-induced immunosuppressed mice.
Conclusion: Sulfur fumigation significantly reduced contents of bioactive ginsenosides in ginseng, leading to dramatic decrease in the systemic exposure of these ginsenosides in the body and detrimental reduction of immunomodulatory effects of ginseng. Our results provided scientific evidences and laid a solid foundation for the needs of thorough evaluation of the significant impact of sulfur fumigation on ginseng and other medicinal herbs.

1. Introduction

Ginseng (Ginseng Radix et Rhizoma, the root and rhizome of *Panax ginseng* C.A. Mey., (Araliaceae)) is one of the ten top-selling natural products worldwide (Qi et al., 2011). It is strongly believed to be a “miracle and cure all” herb against various diseases, which is mainly attributed to its immunomodulatory effects (Liou et al., 2005; Xiang et al., 2008; Zhu et al., 2015a). Modern chemical, pharmacological, and clinical studies have illustrated that ginsenosides, the major ingredients in ginseng, have multiple pharmacological activities, in particular immunomodulatory effects (Han and Rhew, 2013; Lee et al., 2015; Liu et al., 2014; Su et al., 2014; Sun et al., 2007; Wang et al., 2014).

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The traditional post-harvest processing of ginseng is to dry the herb under sunlight or by heating. However, sulfur fumigation, which has been commonly employed for centuries in the food industry worldwide (Carter et al., 2015; Considineand Foyer, 2015; Hayes et al., 2005), has been used in China in recent decades as an alternative processing method for various medicinal herbs, including ginseng, to prevent pest infestation, mold and bacterial contamination, and to provide a favorable whiter appearance (Duan et al., 2016; Jiang et al., 2013; Kan et al., 2011). Our previous study showed that 47% of the tested 38 ginseng samples purchased from various countries were sulfur-fumigated (Li et al., 2012). Although with aforementioned advantages, it has been demonstrated that sulfur fumigation drastically reduced the contents of some major ginsenosides (Jin et al., 2012) and changed the chemical and metabolic profiles of ginsenosides (Cao et al., 2015; Li et al., 2012; Zhu et al., 2015b). To evaluate the beneficial effects of herbs, in addition to chemical profile, the pharmacological activity and pharmacokinetic fate of biological ingredients are also critical prerequisites to interpret and predict a variety of biological events related to the efficacy of medicinal herbs (Li et al., 2011; Pei et al., 2015). In the present study, the effects of sulfur fumigation on the pharmacokinetic fates and immunomodulatory activities of ginseng were investigated. The findings from this study provided important data to support the urgent need for proper evaluation of sulfur fumigation in the post-harvest processing of ginseng and other medicinal herbs.

2. Materials and methods

2.1. Materials and animals

The ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Ro, Rh1, and compound K were purchased from Yuanye Biotechnology Company (Shanghai, China), and Rb3, Rg2, Rh2, F1, F2, protopanaxadiol (PPD) and protopanaxatriol (PPT) were provided by National Institutes for Food and Drug Control (Beijing, China). The structures of the ginsenosides are shown in Fig. 1. 11*α*-*O*-2-Methylbutyryl-12*β*-*O*-acetyltenacigenin B, a structurally similar compound to ginsenoside (Yao et al., 2014), was used as internal standard (IS) for quantitative analysis. Cyclophosphamide (CY), disodium ethylene diamine tetraacetate, heparin, and mannite were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ketamine and xylazine were from Alfasan (Woerden, Holland). All the other chemicals and reagents were of analytical grade.

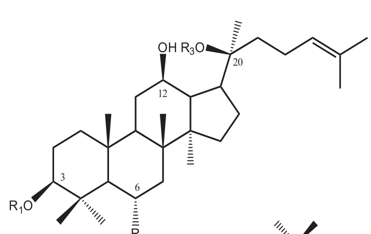
Male Sprague-Dawley rats (220–250 g) and male ICR mice (4–6 weeks old, 18–22 g) were supplied by the Laboratory Animal Service Center, The Chinese University of Hong Kong. Animals were housed under standard conditions of temperature, humidity, and light. Food and water were provided *ad libitum*. The care of animals and all experimental procedures were approved by the Animal Ethics Committee of The Chinese University of Hong Kong. The body weight and general health of the animals were closely monitored during the study.

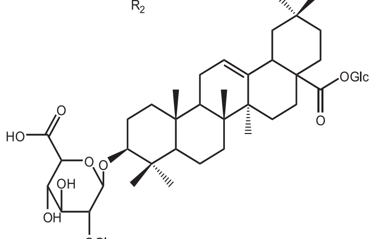
2.2. Instrumentation

Quantitative analysis of ginsenosides in water extracts and plasma samples was performed using an Agilent 6430 triple quadruple mass spectrometer connected to an Agilent 1200 HPLC system (Agilent Technologies, USA). Chromatographic separation was achieved on a Waters Acquity UPLC BEH C_{18}column (1.7 µm, 2.1×100 mm) with an Acquity BEH C_{18} Vanguard Pre-Column (Waters, USA). The mobile phase consisted of (A) acetonitrile containing 0.1% formic acid and (B) H_2O containing 0.1% formic acid. Gradient elution was as follows: 18–30%A (0–5 min), 30–40%A (5–20 min), 40–80%A (20–25 min), 80% A (25–29 min), 80–18%A (29–30 min) and 18%A (30–33 min). The flow rate was 0.4 mL/min. The temperatures of the column oven and autosampler were set at 30 °C and 6 °C, respectively. The injection volume was 5 µL. The mass spectrometer was operated in positive ion mode using an ionspray interface with the following working parameters: capillary voltage, 3500 V; nebulizer gas, 20 psi; gas flow, 8 L/min; gas temperature, 300 °C. Multiple reaction monitoring (MRM) was employed for data acquisition. The detailed MS information including MRM transitions is presented in Table 1. Agilent MassHunter Workstation (B.03.01) was used for HPLC-MS system operation, data acquisition and processing.

2.3. Preparation of non-sulfur fumigated ginseng and sulfur fumigated ginseng extracts

Ginseng (root and rhizome of *Panax ginseng* C.A. Mey., (Araliaceae)) samples were collected from Good Agriculture Practices bases in Jilin, China, and authenticated by Prof. Song-Lin Li. The voucher specimens were kept in the Herbarium of Jiangsu Province Academy of Traditional Chinese Medicine and Jiangsu Branch of China Academy of Chinese Medical Sciences. Sulfur fumigated ginseng (SFG) samples were prepared according to our previous protocol (Li et al., 2012). Briefly, 1 kg of ginseng slices were wetted with 100 mL water and put into a desiccator, where100 g sulfur powder was burned. The desiccator was kept closed for 12 h. After fumigation, the ginseng slices





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Ginsenoside	R1	R2	R3
PPD type			
Rb1	-Glc2-1Glc	-H	-Glc6-1Glc
Rb2	-Glc2-1Glc	-H	-Glc6-1Ara(p)
Rb3	-Glc2-1Glc	-H	-Glc6-1Xyl
Rc	-Glc2-1Glc	-H	-Glc6-1Ara(f)
Rd	-Glc2-1Glc	-H	-Glc
Rh2	-Glc	-H	-H
F2	-Glc	-H	-Glc
Compound K	-H	-H	-Glc
PPD	-H	-H	-H
PPT type			
Re	-H	-OGlc2-1Rha	-Glc
Rf	-H	-OGlc2-1Glc	-H
Rg1	-H	-OGlc	-Glc
Rg2	-H	-OGlc2-1Rha	-H
Rh1	-H	-OGlc	-H
F1	-H	-OH	-Glc
PPT	-H	-OH	-H

Fig. 1. Chemical structures of the 17 ginsenosides measured in rat plasma samples.