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Effects of sulfur-fumigation on the pharmacokinetics, metabolites and analgesic activity of Radix Paeoniae Alba

Ming Kong[a,1], Huan-Huan Liu[a,1], Jie Wu[b], Ming-Qin Shen[c], Zhi-Gang Wang[c], Su-Min Duan[a], Yan-Bo Zhang[d], He Zhu[b,*], Song-Lin Li[a,b,**]

[a] Department of Pharmaceutical Analysis, Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing 210028, PR China

[b] Department of Metabolomics, Jiangsu Province Academy of Traditional Chinese Medicine and Jiangsu Branch of China Academy of Chinese Medical Sciences, Nanjing 210028, PR China

[c] Department of Pharmacology, Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing 210028, PR China

[d] School of Chinese Medicine, The University of Hong Kong, Hong Kong Special Administrative Region

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Compounds:
Oxypaeoniflorin (PubChem CID: 21631105)
Paeoniflorin (PubChem CID: 425990)
Albiflorin (PubChem CID: 51346141)
Gardenoside (PubChem CID: 24721095)

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ABSTRACT

Ethnopharmacological relevance: Radix Paeoniae Alba (*Baishao*, BS), one of the most commonly used traditional Chinese medicinal herbs, has many pharmacological effects including analgesic activity. Previous studies found that sulfur-fumigation, a post-harvest handling process developed to prevent mold contamination of medicinal herbs, altered the quality of BS. However, whether sulfur-fumigation affects the pharmacokinetics, safety and efficacy of BS warrants further investigation.

Aim of the study: To evaluate the feasibility of sulfur-fumigation as a post-harvest handling process of BS from the viewpoints of pharmacokinetics, safety and efficacy.

Materials and methods: The pharmacokinetic behaviors of four active components of BS and one characteristic component of sulfur-fumigated BS (S-BS) were evaluated by high performance liquid chromatography triple quadrupole mass spectrometry (HPLC-TQ-MS/MS). The safety was investigated using ultra high performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS/MS) based metabolomics approach after intragastric (i.g.) administration of non-fumigated BS (N-BS) and S-BS in rats. The analgesic efficacy was compared using hot-plate test in mice, after i.g. administration of N-BS and S-BS, at both high and low dosages.

Results: Systemic exposures of paeoniflorin and oxypaeoniflorin, two analgesic components of BS, were significantly decreased in the S-BS treated group compared to the N-BS treated group, while paeoniflorin sulfonate, one of the sulfur-containing derivatives of S-BS, was detected in all time-points of S-BS treated group with the area under the plasma concentration–time curve (AUC_{0-t}) and the maximum plasma concentration (C_{max}) as high as 7077.06 ± 2232.97 ng/mL*h and 1641.42 ± 634.79 ng/mL respectively, which indicated that sulfur-fumigation altered the pharmacokinetic behaviors of BS. Besides, paeoniflorin sulfonate and its four metabolites with ambiguous toxicities, as well as one endogenous metabolite p-cresol glucuronide, the biomarker of disordered homeostasis of intestinal bacteria and bile acid, were identified as the characteristic metabolites in S-BS administered rats, suggesting that sulfur-fumigation reduced the safety of BS. Furthermore, the analgesic effects at both low and high dosages were decreased in different extent when compared to N-BS administered groups, indicating that sulfur-fumigation weakened the efficacy of BS.

Abbreviations: AUC, area under the plasma concentration–time curve; BS, Radix Paeoniae Alba; CID, collision-induced dissociation; CL, clearance; C_{max}, maximum plasma concentration; FDA, US Food and Drug Administration; HPLC/UV, high performance liquid chromatography/ultra violet detector; HPLC-TQ-MS/MS, high performance liquid chromatography triple quadrupole mass spectrometry; i.g., intragastric; IS, internal standard; MRM, multiple reaction monitoring; N-BS, Non-fumigated Radix Paeoniae Alba; OPLS-DA, orthogonal partial least squared discrimination analysis; PCA, Principal Component Analysis; S-BS, Sulfur-fumigated Radix Paeoniae Alba; SULT, sulphotransferase; $t_{1/2}$, terminal elimination half-life; TCM, traditional Chinese medicine; T_{max}, maximum concentration time; UHPLC-QTOF-MS/MS, ultra high performance liquid chromatography quadrupole time-of-flight mass spectrometry

* Corresponding author.
** Corresponding author at: Department of Pharmaceutical Analysis, Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing 210028, PR China.
E-mail addresses: zhuhev@163.com (H. Zhu), songlinli64@126.com (S.-L. Li).
[1] These authors contributed equally to this work.

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Conclusion: Sulfur-fumigation altered the pharmacokinetics, as well as reduced the safety and efficacy of BS, suggesting that sulfur-fumigation is not recommended for post-harvest handling of BS.

1. Introduction

Sulfur-fumigation has been employed in the near decades to prevent against insects and moulds, and to shorten the drying duration during post-harvest handling process of medicinal herbs (Li et al., 2017). Accumulated studies have indicated that this processing method has impaired the quality of medicinal herbs by reducing the contents of active components (Kong et al., 2014, 2017) and generating new sulfur-containing derivatives of questionable safety (Duan et al., 2016; Pei et al., 2016). To date, the feasibility of using sulfur-fumigation for post-harvest handling of herb is still debatable (Jiang et al., 2013; Kan et al., 2011) and more evidence, beyond quality evaluation, is needed.

Safety and efficacy, two essential aspects of medicinal herbs, are a reflection of the toxicities and activities of any xenobiotics, while the intensities of any toxic effects are related to their concentrations in a bodily fluid (Chaudhari et al., 2016; Shi et al., 2016). Besides, pharmacokinetics, which describes the time course of xenobiotic concentration in a body fluid, is also an important connection between quality and safety/efficacy of medicinal herbs (Ma et al., 2017; Zhu et al., 2011). Thus, feasibility evaluations of sulfur-fumigation in the processing of medicinal herbs from the viewpoints of pharmacokinetics, safety and efficacy should be performed in addition to quality-based investigation.

Routine methods for safety evaluation of xenobiotics are commonly time- and money-consuming (Chaudhari et al., 2016). Moreover, characterized by multi-components against multi-targets, medicinal herbs induced toxicities are much more complex than chemical drugs. So it is a challenge to find an approach to evaluate the safety of medicinal herbs in a convenient and economical manner. Fortunately, a metabolomics approach, which could accurately and quickly provide major insights into the similarities and differences of samples by quantitatively and qualitatively measuring the dynamic change of small molecules, is a powerful tool to identify differential components (Mao et al., 2014), and provides a feasible approach to evaluate the safety of medicinal herbs through exploring characteristic markers, in particular toxic markers (Wang et al., 2017; Zhou et al., 2016).

Radix Paeoniae Alba (*Baishao* in Chinese, BS), derived from the dried root of *Paeonia lactiflora* Pall., is one of the most commonly used medicinal herbs to treat blood deficiency, sallow complexion, menstrual irregularities, spontaneous sweating, night sweating, hypochondriac pain, abdominal pain, spasm and pain of limbs, headache and dizziness (Pharmacopoeia Commission of the People's Republic of China, 2015). In traditional Chinese medicine practice, BS is always used together with other herbs to constitute complex prescription, some of which showed analgesic efficacy in clinic application (Tong et al., 2010). Previous study found that sulfur-fumigation was frequently used in the post-harvest handling of BS, with up to 59% commercial BS samples being sulfur-fumigated (Kong et al., 2014). Besides, sulfur-fumigation could decrease the contents of bioactive compounds in BS and transform monoterpene glycosides (e.g. paeoniflorin), the major components of BS, into their sulfur-containing derivatives (e.g. paeoniflorin sulfonate) (Kong et al., 2014; Li et al., 2009). Recently, the influence of sulfur-fumigation on the pharmacokinetic behaviors of certain chemicals in BS was investigated after intragastric (i.g.) administration of alcohol extracts of BS (Cheng et al., 2010). However, water extraction (decoction), not alcohol extraction, is the main prescription form of traditional Chinese medicine (TCM), so it is much more meaningful to investigate the influence of sulfur-fumigation on pharmacokinetics behaviors of BS water extracts.

In the present study, the influences of sulfur-fumigation on the pharmacokinetics, safety and efficacy of BS were investigated. The findings from this research would provide important data being complementary with those from quality-based studies to comprehensively evaluate the feasibility of sulfur-fumigation as a post-harvest handling process of BS.

2. Materials and methods

2.1. Reagents and animals

Reference compounds of oxypaeoniflorin and paeoniflorin were purchased from Shanghai Sunny Biotech Co., Ltd. and Chengdu Preferred Biological Technology Co., Ltd, respectively. Paeoniflorin sulfonate and gardenoside (internal standard, IS) were provided by Shanghai U-sea Biotech Co., Ltd and the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Albiflorin and benzoypaeoniflorin were isolated from BS in our lab and confirmed by MS and NMR analysis. The purities of all compounds were determined to be higher than 95% by high performance liquid chromatography/ultra violet detector (HPLC/UV) analysis. Acetonitrile and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany), and formic acid (HPLC grade) was purchased from ROE (USA). Deionized water was prepared in-house with Millipore (Millipore, MA, USA).

Male Sprague-Dawley rats (220–250 g) and male Kunming mice (18–22 g) were supplied by Shanghai Slac Laboratory Animal Co., Ltd., China. All animals were housed under controlled temperature 25 ± 1 °C, relative humidity $40 - 70\%$. The animal care and use were in accordance with the Regulations of Experimental Animal Administration issued by the Ministry of Science and Technology of China, and the experimental protocols were approved by the Animal Affairs Committee of Jiangsu Province Academy of Traditional Chinese Medicine and Jiangsu Branch of China Academy of Chinese Medical Sciences.

2.2. Preparation of N-BS and S-BS extracts

Fresh Radix Paeoniae Alba samples (Batch No. 20141010) was collected from Bozhou (Anhui Province, China), one of its indigenous cultivating regions in China. All samples were authenticated by Prof. Song-Lin Li to be the root of *Paeonia lactiflora* based on the morphological and histological features according to the monograph of BS documented in Chinese Pharmacopoeia (Pharmacopoeia Commission of the People's Republic of China, 2015). Non-fumigated BS sample (N-BS, JSPACM-1-K-31) was prepared according to Chinese Pharmacopoeia (Pharmacopoeia Commission of the People's Republic of China, 2015). In brief, the fresh Radix Paeoniae Alba samples were immersed in hot water (about 90 °C) for 20 min, peeled off the skins, cut into slices with the thickness of about 0.5 cm and the diameter of about 1.5–2 cm, and then dried in oven at 40 °C to get N-BS samples. The sulfur-fumigated BS sample (S-BS, JSPACM-1-K-33) was prepared from N-BS (JSPACM-1-K-31) according to our previous protocol with minor modifications which imitated the sulfur-fumigation process of BS commonly utilized by the wholesalers (Kong et al., 2014). Briefly, N-BS samples were wetted with water (10:1, w/w), put into a desiccator, sulfur-fumigated for 26 h and then dried in oven at 40 °C to get S-BS samples. The voucher specimens of the N-BS and S-BS samples were deposited in Department of Metabolomics, Jiangsu Province Academy of Traditional Chinese Medicine and Jiangsu Branch of China Academy of Chinese Medical Sciences. The contents of paeoniflorin albiflorin, gallic acid,