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Toxicology

Health risk assessment of arsenic in Realgar and NiuHuangJieDu Tablets based on pharmacokinetic study

Xiao Wu, Shanhu Wu, Yuexin Liu, Rong Guan, Fangmei Liang, Min Song*, Taijun Hang*

Key Laboratory of Drug Quality Control and Pharmacovigilance, Ministry of Education, Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing 210009, China

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ABSTRACT

NiuHuangJieDu Tablets (NHJDT), a popular realgar (As_4S_4) containing patented traditional Chinese medicine (TCM), is widely used in the treatment of acute tonsillitis, pharyngitis, periodontitis and mouth ulcer. However, arsenic is considered as one of the most toxic elements, leading to growing concerns about the quality and safety of realgar-containing TCMs recently. In this study, health risk assessment of arsenic in realgar and NHJDT was conducted through oral administration of both substances to rats with single and multiple doses, respectively. The total blood arsenic concentration was used as the health risk indicator and determined by hydride generation-atomic fluorescence spectrometry after modified Kjeldahl digestion, and then applied to the pharmacokinetic study. For single oral dose study in rats, the low, medium, and high doses of realgar and NHJDT were set equivalent to 1, 5 and 20 times the human therapeutic dose (1.3 mg realgar/kg), respectively. Multiple doses were given at low and high dose levels every 12 h for seven consecutive days, respectively. Significant differences in the total blood arsenic pharmacokinetic profiles were observed between the corresponding realgar and NHJDT groups. These results indicated that NHJDT significantly reduced the total blood arsenic exposure present in realgar, and the detoxification mechanism might be attributed to herb–herb interactions in NHJDT. However, the accumulation of blood total arsenic was significant due to the long elimination half-life and high accumulation index in both realgar and NHJDT groups. Therefore, the potential health risk of arsenic caused by the administration of realgar-containing TCMs should be taken into account for excessive or long-term medication. Precautions should be taken for the clinical application of realgar-containing TCMs.

1. Introduction

Traditional Chinese medicines (TCMs) have been widely used for hundreds of years and spread all over the world. In some TCMs, minerals, metals or metalloids are deliberately combined with herbs for specific curative purposes. Realgar, for example, an arsenic-containing mineral material in the form of As_4S_4 listed in the current Chinese Pharmacopoeia, has been incorporated in some of the traditional Chinese medicines for hundreds of years up till now [1]. These realgar-containing TCMs have shown beneficial effects on itch-relief, putrefaction removal and dampness elimination [2]. In recent years, realgar-containing TCMs have also been proved to have remarkable therapeutic efficacy in hematological malignancies and acute promyelocytic leukemia [3,4].

According to the 2015 edition of the Chinese pharmacopeia, there are 37 (2.48%) types of TCMs containing realgar among the listed total of 1493. NiuHuangJieDu Tablets (NHJDT) is one of the most common and popular over-the-counter realgar-containing TCM with a long history of clinical application [5]. It is officially listed in the current Chinese Pharmacopeia with eight herbal components including realgar (26.3 mg per tablet or 6.4% weight percent of the total prescription), Niu Huang (*Bovis Calculus*), Huang Qin (*Scutellariae Radix*), Da Huang (*Rhei Radix et Rhizoma*), Shi Gao (*Gypsum Fibrosum*), Jie Geng (*Platycodonis Radix*), Bing Pian (*Borneolum Syntheticum*), and Gan Cao (*Glycyrrhizae Radix et Rhizoma*). It has antipyretic and detoxicate effects and is widely used for the treatment of sore throat and gingivitis [6].

However, arsenic is considered as one of the most toxic elements in the environment and is well-known for its acute and chronic toxicity [7]. In ancient times, “arsenic” and “poison” were almost synonymous. World Health Organization’s International Agency for Research on Cancer (IARC) classified inorganic arsenic as carcinogen to humans for the risk of skin and lung cancers, cardiovascular disease, peripheral neuropathy, bone marrow depression, gastrointestinal symptoms and other serious health problems [8,9]. Medicines and healthcare products regulatory agency (MHRA) has issued alerts about the TCMs for extraordinary high arsenic contents, which raises questions about the

* Corresponding authors.
E-mail addresses: cqsongmin@sina.com (M. Song), hangtj@cpu.edu.cn (T. Hang).

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safety and usage of realgar-containing TCMs among the public [10]. Although realgar is relatively insoluble in gastro-intestinal fluids through *in vitro* determination, and the bioavailability of realgar from TCMs are extremely low [11,12], cases of arsenic poisoning from taking realgar and realgar-containing TCMs have often been reported [13–15]. Attention has been mainly focused on the bioavailability of arsenic in traditional medicines *in vitro* [16–18], but there still lacks information regarding the behavior of arsenic in human body. Hence, the *in vivo* arsenic health risk assessment of realgar-containing TCMs is strongly needed.

Blood arsenic concentration is a reliable health risk indicator for assessing exposure since only the bioavailable portion of arsenic can be absorbed by humans and transported into the blood stream [19]. Pharmacokinetics, an important safety evaluation method, has been applied in the human health risk assessment [20]. The pharmacokinetic profiles of arsenic trioxide (As_2O_3) in primary hepatocarcinoma and acute promyelocytic leukemia patients have been reported for safety evaluation [21,22], but the pharmacokinetic data of realgar and realgar-containing TCMs are still rarely reported [23,24]. Although there have been investigations of pharmacokinetics of arsenic species in rat plasma after single oral administration of realgar-containing TCMs of XiaoErZhiBao pills and NiuHuangJieDu tablets [23,24], the safety of realgar-containing TCMs cannot be accurately evaluated in plasma since over 99% of total arsenic significantly accumulated in red blood cells (RBCs) of rats in the form of hemoglobin complexed with dimethylarsinous acid (DMA) regardless of administration of arsenic species [25–27]. Therefore, the use of arsenic species in plasma to evaluate the safety of realgar-containing TCMs is not scientifically effective and the pharmacokinetics of total arsenic in the whole blood is strongly needed.

For arsenic determination, inductively coupled plasma-mass spectrometry (ICP-MS) and hydride generation-atomic fluorescence spectrometry (HG-AFS) are commonly employed because of their high sensitivity, good reproducibility and wide linear range [28]. AFS, a suitable alternative to ICP-MS on detection limits, presents the benefits of lower acquisition and running cost, shorter warm-up times and easy handling. In the present study, a HG-AFS method was established after modified fast Kjeldahl digestion for safety assessment of total blood arsenic in rats with single and multiple oral doses of reaglar and NHJDT, respectively. This study aims to provide scientific reference for the safety assessment and clinical application of realgar-containing TCMs.

2. Materials and methods

2.1. Materials and reagents

Water lapping realgar (Batch No.160319, purity of 92.86%) was purchased from Sanmenxia Yuhuangshan Pharmaceutical CO., Ltd. (He'nan, China). NHJDT (Batch No.151221090, 0.27 g/tablet) was obtained from Beijing Tongrentang Technologies CO., Ltd. (Beijing, China). Arsenic trioxide reference standard was provided by National Institutes for Food and Drug Control (Beijing, China). Dimethylarsenic acid (DMA) was obtained from Huamaike Biotechnology Co., Ltd. (Beijing, China). Thiourea, ascorbic acid and potassium borohydride were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium carboxymethylcellulose, potassium hydroxide and potassium sulfate were purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Heparin sodium was from Biosharp Co., Ltd. (Hefei, China). Hydrochloric acid, nitric acid and sulphuric acid (Sinopharm Chemical Reagent Co., Ltd.) were of guaranteed grade. All the other chemicals were of analytical reagent grades. Water was purified with a Millipore Milli Q-Plus purification system (Millipore, MA, USA). All the glass wares were soaked with 20% HNO_3 solution for at least 24 h and then cleaned and rinsed thoroughly with purified water. An As (V) reduction reagent solution was freshly prepared as a mixture of 10% (w/v) thiourea and 10% (w/v) ascorbic acid in water.

Table 1
AFS parameters for arsenic HG determination.

Hydride generation	Values
Acid solution	1.85% HCl (w/v), 15 mL/min
Reducing agent	1.0% KBH_4 in 0.2% KOH (w/v), 15 mL/min
AFS system	
Lamp	Hollow cathode arsenic lamp, 193.7 nm
PMT voltage	270 V
Primary current	70 mA
Carrier gas	Argon, 700 mL/min
Auxiliary Gas	Argon, 300 mL/min
Atomizer temperature	200 °C
Atomizer height	7 mm
Sampling time	8 s
Injection time	21 s
Analysis period (s)	17 s
Delay period (s)	2 s
Measurement mode	Peak area

2.2. Apparatus and analytical methods

Total blood arsenic was determined by HG-AFS (AF-610D, Rayleigh, Beijing) after modified fast Kjeldahl digestion. The instrument was set same as reported [24,29] with the key parameters tested as follows. Hydride generation was achieved by pumping 1.0% KBH_4 (stabilized in 0.2% KOH, w/v) and 1.85% HCl (w/v) solutions each with a flow rate of 15 mL/min. The produced arsenic hydride was separated by argon gas flow in gas-liquid separator and carried to quartz atomizer warmed by infrared radiation and then atomized in argon-hydrogen flame to generate signals of AFS. Hollow cathode lamp and PMT voltage were set at 270 V and 70 mA, respectively. The optimum AFS conditions are summarized in Table 1 (Supplementary S1).

2.3. Preparation of calibration standards

As(III) stock solution (1.0 mg/mL calculated on As base) was prepared by dissolving an accurately weighed amount of As_2O_3 standard substance about 0.13 g in 1 mL of 2.5 mol/L NaOH and then diluted with 0.3 mol/L HCl solution in 100 mL volumetric flask and stored in refrigerator at 4 °C before use.

Series of As(III) working standard solutions in the linear range from 0 to 200 ng/mL (0, 10, 20, 50, 80, 100, 150 and 200 ng/mL) were freshly prepared by appropriate dilution of the stock solution in 25 mL volumetric flasks with 1.85% HCl (w/v) solution after the addition of 2.5 mL As(V) reduction reagent solution.

2.4. Total blood arsenic determination

The blood samples were pretreated with a modified fast Kjeldahl digestion as follows.

An aliquot of 0.1 g whole blood sample, accurately weighed and placed in a 50 mL beaker with a watch glass cover, was mixed with 0.3 g K_2SO_4 and 3 mL H_2SO_4-HNO_3 (2:1) and left stand overnight (Supplementary S2). Then the mixture was digested by heating on hotplate of 200–250 °C for about 1 h until a clear and transparent solution was produced. After cooling to room temperature, the solution was carefully mixed with 1 mL of water, and boiled gently for another 10 min to decompose and evaporate the residual HNO_3.

The digested solution was then cooled to room temperature and quantitatively transferred to a 25 mL volumetric flask with 1.85% HCl (w/v) solution and diluted to volume after the addition of 2.5 mL As (V) reduction reagent solution. The resulting test solution was left standing at room temperature for about 30 min before the determination to ensure all the arsenics were thoroughly converted to As (III) by thiourea