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Estimation of bioaccessibility and potential human health risk of mercury in Chinese patent medicines

Lihong Liu[1], *Yu Zhang*[2], *Zhaojun Yun*[1], *Bin He*[1,*], *Guibin Jiang*[1]

1. *State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Centre for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China. E-mail: lhliu@rcees.ac.cn*

2. *Wangjing Hospital, China Academy of Chinese Medical Sciences, Beijing 100102, China*

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ABSTRACT

Mercury (Hg), mainly in cinnabar species, has been used in medicine for thousands of years in China, and worldwide concern has been raised on its toxicity. In this work, the amount of bioaccessible mercury in 16 Chinese patent medicines (CPMs) was measured by using an *in vitro* simulated digestion system, consisting of simulated gastric and intestinal fluid, to investigate the bioavailability of mercury in CPMs and evaluate its potential risk to human health. Total mercury and mercury in the gastrointestinal extracts were measured by inductively coupled plasma mass spectrometry (ICP-MS). The levels of total Hg in 16 CPMs ranged from not detected to 11.89 mg/g, with a mean value of 1.13 mg/g, while the extractable Hg ranged from not detected to 4.37 μg/g, with a mean value of 0.42 μg/g. Mercury bioaccessibility varied significantly in the investigated CPMs, depending on the ingredient. Compared to the CPMs without cinnabar (2.5%–30.9%), the percentage of mercury in the gastrointestinal supernatants for CPMs with cinnabar was quite a bit lower (0.037%). By comparing with the Food and Agricultural Organization/World Health Organization Joint Expert Committee on Food Additives (FAO/WHO) safety guideline, the average daily intake dose (ADD) of Hg in the medicines was then calculated to access the risk of mercury to human health from taking CPMs.

Introduction

Traditional Chinese medicine (TCM) has been widely used in many eastern countries for thousands of years and spread all over the world. The U.S. botanical market is a booming industry, amounting to about 1.5 billion dollars in 1995 and probably growing at about 15% a year (Marwick, 1995). Chinese patent medicine (CPM) is one type of TCM, which is composed of different ingredients, such as prescriptive botanicals, animal tissues or minerals. CPMs may come in different forms such as pills, powders, syrups, liquids, tablets, granules and capsules. As alternative or complementary medicine, even though TCMs are perceived to be natural and thus harmless by many consumers, problems might arise because of contamination, the lack of adequate regulations and the pharmacological complexity of herbal products (Ernst, 1998, 2002). Along with the popularity of TCM, more and more concerns about the quality and safety of the medicines have been raised nowadays (Ernst, 1998, 2002; Kim et al., 2013). For instance, heavy metals, such as mercury, lead, arsenic, and cadmium, have been previously reported to be present in TCMs (Ting et al., 2013). In the 251 CPMs obtained from California herbal retail stores, 35 contained an average of 1046 μg/g mercury, 36 contained an average of 14.6 μg/g arsenic, and 24 contained at least 10 μg/g lead (Ko, 1998). The

* *Corresponding author*. E-mail: bhe@rcees.ac.cn (Bin He).

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concentrations of toxic heavy metals in 42 of 2080 TCMs were found to exceed Singapore's legal limits, including 28 for mercury, 6 for arsenic, and 8 for lead (Ernst, 2002; Koh and Woo, 2000). Such issues were also reported in Europe (Melchart et al., 1999; Zhang et al., 2012) and Japan (Itoh et al., 1995).

Mercury is considered one of the most toxic elements in the environment and it can cause many diseases and side effects in human beings, especially to the nervous system. Therefore, CPMs containing Hg are expected to pose significant health risks. Cases of mercury poisonings from taking CPMs have been reported (Ernst and Coon, 2001). Mercury in CPMs can originate from the accumulation of Hg in the raw materials and/or inadvertent contamination during the production process (Zhang et al., 2012). Besides contamination, deliberate addition for specific curative purposes is another reason for the high concentration of Hg in CPMs. Cinnabar, a naturally occurring mineral containing more than 96% mercury sulfide (HgS), has been widely used in CPMs for more than 2000 years due to its sedative and hypnotic function (Lu et al., 2011). There are 63 prescriptions containing cinnabar among the 1060 Chinese medicinal prescriptions recorded in the latest Chinese pharmacopeia (Pharmacopeial Committee of China, 2010).

Generally, it is assumed that 100% of the Hg is absorbed by the human body and therefore, the total Hg level is usually taken as the most important criteria for evaluation of the toxicity and health impact of taking medicines. Human health risk associated with exposure to mercury by taking CPMs has been evaluated in several previous studies by estimating the total amount of Hg ingested (Chui et al., 2013; Liu et al., 2012). However, only the bioavailable portion of Hg can be absorbed by humans and transported into the blood stream, freely cross cellular membranes and redistribute around the body. Therefore, the potential toxicity could be overestimated in these studies without considering the bioavailability of Hg. The toxicity of Hg in CPMs depends not only on the total concentration, but also on its bioavailability, which can be absorbed and finally reach systemic circulation. Therefore, determining the amount of bioavailable Hg in the CPMs is necessary for appropriately assessing the health risk of consuming CPMs.

Due to the disadvantages of *in vivo* methods for bioavailability determination, *e.g.*, being time consuming, expensive, and complicated, an *in vitro* method for determining bioaccessibility (fraction dissolved in the digestive system and potentially available for absorption) has been commonly used to estimate the bioavailability of metals in real samples (Koch et al., 2007). The *in vitro* extraction can be strongly affected by various conditions, such as temperature, pH, agitation and extract composition, which should be similar to those in the human body during digestion. Simulated body fluid (SBF) tests have been used to study the bioaccessibility of several metals in food (Juhasz et al., 2006; Moreda-Pineiro et al., 2011), soil (Kientz et al., 2003), and sediment (Semple et al., 2004), in which the bioaccessible fraction was extracted by using simulated gastric fluid (SGF) or SGF and simulated intestinal fluid (SIF) together. Cabanero et al. (2004) have used the SBF test to study the Hg bioaccessibility in fishes. Their results showed that the proportion of bioaccessible Hg in fishes ranged from 9%–17%, depending on the species. However, to the best of our knowledge, none of the previous studies investigated the bioaccessibility of Hg in CPMs.

In this work, we attempted to determine the total and bioaccessible amount of Hg in 16 commonly used CPMs. In order to study the bioaccessibility of Hg in the CPMs, an *in vitro* SBF test, including SGF and SIF phase extractions, were performed to simulate the gastric and intestinal digestion process in the human body. The total and SGF/SIF extractable Hg was then determined by inductively coupled plasma mass spectrometry (ICP-MS). Finally, the average daily intake dose (ADD) was calculated to assess the potential health risk of taking these CPMs.

1. Materials and methods

1.1. Chemicals and reagents

Mercury stock standard solution (1000 mg/L as Hg) was prepared by dissolving mercury chloride (≥99.5%, Beijing Chemical Reagents Company, Beijing, China) in deionized water and then stored in the dark at 4°C. Au solution (1000 mg/L Au) was purchased from Inorganic Ventures (Christiansburg, USA). Hg working solutions were prepared by diluting a certain amount of the stock standard solution in 3% HNO_3 (65%, Merck, Darmstadt, Germany) containing 100 ng/mL Au. All other chemicals used were analytical or higher grade. De-ionized water (18.2 MΩ cm) was made by a Milli-Q Advanced A10 ultrapure water system (Millipore, Bedford, USA).

Both SGF and SIF were prepared according to the procedure described in the U.S. Pharmacopeia (United States Pharmacopeial Convention, 2004). The SGF was prepared by dissolving 3.2 g of pepsin (from porcine gastric mucosa, 920 units/mg protein, Sigma-Aldrich Co. LLC, USA) and 2 g of NaCl in 7.0 mL of concentrated HCl. Then the mixture was diluted to 1 L with de-ionized water, and the pH of the final solution was about 1.2. The SIF was prepared by dissolving 6.8 g KH_2PO_4 in 250 mL de-ionized water, adding in sequence 77 mL of 0.2 mol/L NaOH, 500 mL of de-ionized water, and 10.0 g of pancreatin (from porcine pancreas, 8 × USP specifications, Sigma-Aldrich Co. LLC, USA). Then the mixture was diluted to 1 L with de-ionized water, and the pH of the final solution was about 6.8.

1.2. ICP-MS system

An Agilent 7500ce ICP-MS (Agilent Technologies, USA) was used for the Hg quantification. A Babington nebulizer was fitted on the double-pass spray chamber. The ICP-MS instrument performance conditions, including nebulizer gas flow rate, carrier gas flow rate, makeup gas flow rate, and lens voltage, were tuned and optimized daily. The optimized parameters are shown in Table 1. In order to eliminate memory interference effects of Hg in the ICP-MS system, an aliquot of Au stock solution was added to all blanks, calibration standards and samples to form a final concentration of 100 ng/mL Au in all the solutions (U.S. Environmental Protection Agency, 1994). The concentrations of Hg in the microwave-digested samples, gastric phase and intestinal phase extracted samples were analyzed by using the external calibration method. Bismuth (Bi) was used as an internal standard to correct for changes caused by the variation of matrix components of different samples and the instrument drift during measurement.