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Mercury sulfides are much less nephrotoxic than mercury chloride and methylmercury in mice

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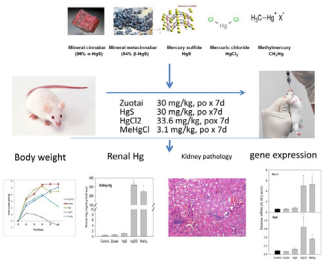
HIGHLIGHTS

- Mercury sulfides differ from $HgCl_2$ and MeHg in producing kidney injury.
- Renal Hg after mercury sulfides is much less than after $HgCl_2$ and MeHg.
- Renal Kim-1 and Ngal are differentially expressed between HgS, $HgCl_2$ and MeHg.
- Mercury sulfides differ from $HgCl_2$ and MeHg in renal transporter gene expression.
- Mercury sulfides in traditional medicines: chemical form matters.

GRAPHICAL ABSTRACT



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ABSTRACT

Mercury sulfides (α-HgS, β-HgS) are frequently included in traditional medicines. Mercury is known for nephrotoxicity, their safety is of concern. To address this question, mice were orally administrated with Zuotai (54% β-HgS, 30 mg/kg), α-HgS (HgS, 30 mg/kg), $HgCl_2$ (33.6 mg/kg), or MeHgCl (3.1 mg/kg) for 7 days, and nephrotoxicity was examined. Animal body weights were decreased by $HgCl_2$ and to a lesser extent by MeHg, but unaltered after Zuotai and HgS. $HgCl_2$ and MeHg produced renal tubular vacuolation, interstitial inflammation and cell degeneration with protein cysts in the tubular lumen, while these pathological lesions were mild in Zuotai and HgS-treated mice. Electron microscopy showed that $HgCl_2$ and MeHg produced spotted swelling endothelium reticulum, while these lesions were mild or absent in Zuotai and HgS-treated mice. Renal Hg contents reached 250–300 ng/mg kidney in $HgCl_2$ and MeHg groups as compared to 2–3 ng/mg in Zuotai and HgS groups. The expression of kidney injury biomarkers, kidney injury molecule-1 (Kim-1) and neutrophil gelatinase-associated lipocalin (Ngal), were increased after $HgCl_2$ and MeHg, but unaltered after Zuotai and HgS. The expression of renal influx transporters Oat3 and Oatp4c1 was decreased, while the expression of renal efflux transporter such as Mrp2, Mrp4, and Mate2 was increased following $HgCl_2$ and MeHg. These gene expressions were unchanged after Zuotai and HgS. In summary, both α-HgS and β-HgS are less nephrotoxic than $HgCl_2$ and MeHg, indicating that chemical forms of mercury are a major determinant of mercury disposition and toxicity.

Abbreviations: Kim-1, Kidney injury molecule 1; Ngal, Neutrophil gelatinase associated lipocalin; Oat3, Organic anion transporter 3; Oatp4c1, Organic transporting peptide 4c1; Mrp2, Multidrug resistance-associated protein 2; Mate2, Multidrug resistance-associated protein 2; MeHg, Methylmercury.

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1. Introduction

In traditional Indian Ayuvedic medicines, Chinese medicines and Tibetan medicines, mercury sulfides are frequently included in the herbo-metallic preparations for treatment of various diseases for thousands of years. Nowadays, in Pharmacopeia of China (2015), over 30 recipes contain cinnabar (α-HgS); in Indian Ayurvedic medicine, *Rasasindura* that is primarily composed of mercuric sulfide (α-HgS and metacinnabar, β-HgS), is included in over 20 recipes (Kamath et al., 2012); in Tibetan medicine, *Zuotai* that is mainly composed of β-HgS, is included in a dozen of Tibetan remedies (Kan, 2013; Li et al., 2015). Mercury sulfides-based traditional medicines are used for acute brain emergencies such as stroke, brain trauma, neuroinflammation, and high fever, but also used to treat chronic ailments like syphilis, pneumonia, insomnia, nervous disorders, paralysis of the tongue, and even used as a rejuvenator to improve strength, stamina and energy (Kamath et al., 2012; Kan, 2013; Li et al., 2015; Pharmacopoeia of China, 2015). Mercury is a toxic heavy metal, and public concerns on the safety of these mercury-containing traditional medicines are increasing. For example, the allowable amounts of cinnabar in Chinese medicines have been dramatically decreased by as much as 65%, from a daily allowable dose of 0.3–1.5 g in 1977 to 0.1–0.5 g (Pharmacopoeia of China, 2015; Zhou et al., 2009). However, the total mercury contents in these traditional medicines are still thousands of folds higher than allowable environmental exposure levels. An opposing opinion held that metal-containing traditional medicines, such as Ayurvedic medicines, are not necessarily toxic at safe therapeutic levels (Mao and Desai, 2009). It was strongly recommended that well-designed studies are needed to address the true risk of the use of HgS-containing medicines (Kamath et al., 2012; Liu et al., 2008; Mao and Desai, 2009).

It should be noted that only mercury sulfides (α-HgS, β-HgS), but not mercury chloride ($HgCl_2$) and methylmercury (MeHg), that are included in traditional remedies, and their chemical structures are quite different (Fig. 1). When discussing mercury toxicity, the chemical forms of mercury must be distinguished (Klaassen, 2006). Kidney is the major target organ of mercury toxicity (Klaassen, 2006). We have previously shown that cinnabar (96% α-HgS) is different from $HgCl_2$ and MeHg in producing kidney injury in mice (Lu et al., 2001a,b) and in rats (Shi et al., 2011). However, whether the same scenario applies to Zuotai that contains 54% β-HgS (Xia et al., 2010; Li et al., 2015) is not known. In addition, the differential effects of Zuotai and pure HgS from $HgCl_2$, MeHg on renal transporters and renal Hg accumulation are not known. The goal of the current study was to compare the nephrotoxicity potentials of Zuotai (54% β-HgS) and pure form of mercury sulfide (α-HgS, HgS) with mercury chloride ($HgCl_2$) and organic methylmercury (MeHg) in mice, focusing on renal mercury accumulation, ultrastructural changes, and gene expressions related to renal toxicity and transporters.

2. Materials and methods

2.1. Chemicals and animals

Zuotai was provided by the Northwest Plateau Institute of Biology of Chinese Academy of Sciences. The pure form of cinnabar (α-HgS), $HgCl_2$ and MeHgCl were from Sigma Chemical Company (St. Louis, MO). Other reagents were of reagent grade.

Adult Kunming outbred mice, 25 ± 2 g, male and female, were purchased from the Laboratory Animals Center of the Third Military Medical University (Chongqing, China). Mice were maintained in a room at 22 ± 2 °C with a 12 h light-dark cycle, and had free access to standard rodent chow and water. They were allowed to acclimate for at least seven days prior to experiment. All the experiments were carried out in full compliance with the WHO Guidance of Humane Care and Use of Laboratory Animals, and approved by the Animal Care and Use Committee of Zunyi Medical College.

2.2. Experimental design

Adult mice were divided randomly to five groups, 6–8 mice per group. Mice were orally given Zuotai (54% β-HgS, 30 mg/kg for 7days). A 5-fold of clinical dose of Zuotai (Li et al., 2014) was used to study nephrotoxicity. For comparison, α-HgS (HgS, 30 mg/kg), $HgCl_2$ (33.6 mg/kg, equivalent Hg as α-HgS), MeHg (3.1 mg/kg, 1/10 Hg of α-HgS), or distilled water (10 ml/kg) was gavaged daily for consecutive 7 days. Animals were closely monitored throughout the entire experiment period and body weights were recorded daily. At the end of experiment, animal body weights and kidney weights were recorded and blood, kidneys were collected for further analysis.

2.3. Histological evaluation

A portion of the kidney was placed in 10% neutral formalin. Fixed tissues were paraffin embedded, sectioned at 6 μm and stained with hematoxylin and eosin (H&E) and examined with Leica microscope. DP image software was used to capture images.

2.4. Ultrastructural analysis

The kidneys were quickly removed, and cut in small pieces ($1 \times 1 \times 1\ mm^3$) on ice pad. The kidney tissues were pre-fixed immediately in 3% glutaraldehyde for 36 h, and dipped in the fixative solution of 1% osmium tetroxide, and then processed with standard sample preparation for electromicroscopy (passed the graded alcohol for dehydration, embedded in spur epoxy resin, and cut thin slice of 70 nm). The section was stained with uranyl acetate and electronic lead nitrate for transmission electron microscope (Hitachi H-7650) examination.

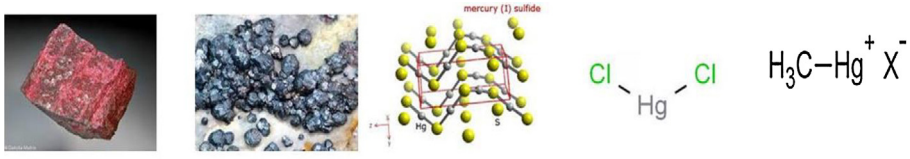

Fig. 1. Zuotai and cinnabar are structurally different from $HgCl_2$ and MeHg.