Original Research

Realgar, cinnabar and An-Gong-Niu-Huang Wan are much less chronically nephrotoxic than common arsenicals and mercurials

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Abstract

Realgar (As_4S_4) and cinnabar (HgS) are frequently included in traditional Chinese medicines and Indian Ayurvedic medicines. Both As and Hg are well known for toxic effects, and their safety is of concern. The aim of this study was to compare chronic nephrotoxicity of An-Gong-Niu-Huang Wan (AGNH), realgar and cinnabar with common arsenicals and mercurials. Mice were orally administrated with AGNH (3 g/kg, 6-fold of clinical dose), cinnabar (0.3 g/kg, amount in AGNH) and realgar (0.3 g/kg, amount in AGNH), $HgCl_2$ (0.118 mmol/kg, 1/10 of cinnabar), MeHg (0.012 mmol/kg, 1/100 of cinnabar), $NaAsO_2$ (As^{3+} 0.028 mmol/kg, 1/100 of realgar) or Na_2HAsO_4 (As^{5+} 0.056 mmol/kg, 1/50 of realgar), daily for six weeks, and nephrotoxicity was examined. Animal body weights were decreased by MeHg and $HgCl_2$. Blood urea nitrogen and creatinine levels were elevated by MeHg. Renal pathology was severe in the MeHg and $HgCl_2$ groups, moderate in the arsenite, arsenate and realgar groups and mild in the cinnabar and AGNH groups. Renal Hg accumulation in the MeHg and $HgCl_2$ groups was 50–200 folds higher than the cinnabar group. Expressions of metallothionein-1 and heme oxygenase-1, biomarkers for metal toxicity, were increased 2–5 folds by arsenite, arsenate, MeHg and $HgCl_2$, but not by realgar, cinnabar and AGNH. The chemokine and glutathione-*S* transferase-α4, markers for inflammation, were also increased by MeHg and $HgCl_2$. Expressions of cell adhesion gene S100a9 and E-cadherin were altered by $HgCl_2$, arsenite and realgar. Taken together, chemical forms of mercury and arsenic are major determinants in their disposition and toxicity.

Keywords: realgar, cinnabar, MeHg, $HgCl_2$, arsenite, arsenate, chronic nephrotoxicity

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Introduction

Mineral arsenicals such as realgar (90% of As_4S_4) and mercurials such as cinnabar (96% of HgS) have been used in traditional Chinese medicines[1–3] and Indian Ayurvedic medicines,[4,5] and claimed to have therapeutic effects. Metals in traditional medicines raise escalating public concerns.[5–7] These traditional medicines are forbidden in the USA or European market, because contents of arsenic and mercury are over the allowable limits.[2,3] The Chinese Pharmacopeia Committee has reduced the allowable arsenic and mercury contents in traditional Chinese medicines by as much as 65%,[1] but these realgar- and cinnabar-containing remedies are still thousands of folds over the allowable limits. There is a general perception that any intentional use of such metal-containing remedies is unacceptable. An opposing opinion held is that these mineral arsenicals and mercurials used in traditional medicines are not necessarily toxic, as they are in the forms of sulfides such as mercury sulfide (cinnabar) and arsenic sulfide (realgar), and these sulfides are less toxic.[1–3,8,9]

Realgar is also called red arsenic due to a deep red color, or *Xionghuang*. It contains >90% tetra-arsenic tetra-sulfide and arsenic disulfide (As_4S_4, As_2S_2). Cinnabar is a naturally occurring mineral with mercury in combination with sulfur, is red in color and is therefore called red mercury sulfide, *Zhu Sha* or China Red. Both realgar and cinnabar are widely included in traditional medicines for both external and internal uses.[1,4,10] For example, the famous traditional Chinese medicine An-Gong-Niu-Huang Wan (AGNH) contains 10% realgar and 10% cinnabar and is effective for acute and chronic brain disease including viral encephalitis, cerebral stroke, cerebral hemorrhage and severe cranio-cerebral trauma as a complementary and alternative medicine.[11]

In recent years, the toxicity effects from realgar- and cinnabar-containing traditional medicines have been reported, such as realgar-induced renal injury[12] and cinnabar-induced kidney toxicity.[9,13] Thus, the questions

became 'Is AGNH safe?' 'Should realgar and cinnabar be removed from AGNH?' It is strongly suggested that well-designed investigations are needed to evaluate the true risk of using metal-containing medicines.[14]

To address the first question, this study was designed to compare chronic toxic effects of realgar, cinnabar and AGNH with common mercurials ($HgCl_2$ and MeHg) and arsenicals (arsenite and arsenate) in mice, focusing on renal injury. The results clearly showed that AGNH, cinnabar and realgar were much less toxic than arsenite, arsenate, MeHg and $HgCl_2$, indicating that the chemical form of metals underlie their disposition and toxicity potentials.

Materials and methods

Chemicals and animals

AGNH (3 g per pill), realgar (90% As_4S_4) and cinnabar (96% HgS) were purchased from Guiyang De-Chang-Xiang Drug Company (Guizhou, China). $NaAsO_2$, $Na_2HAsO_4 \bullet 7H_2O$, MeHg and $HgCl_2$ were from Sigma Chemical Company (St Louis, MO, USA). 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 $22 \pm 2^{\circ}C$ 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 the experiment. All the experiments were carried out in full compliance with the WHO Guidance of Humane Care and Use of Laboratory Animals.

Experimental design

Adult mice were divided randomly into eight groups, 6–7 mice per group. Mice were orally given distilled water (Control), AGNH (3 g/kg, 6-fold of clinical dose), realgar (0.3 g/kg, equal to 2.8 mmol As/kg), cinnabar (0.3 g/kg, equal to 1.18 mmol Hg/kg), sodium arsenite (0.028 mmol/kg, 1/100 of realgar), sodium arsenate (0.056 mmol/kg, 1/50 of the realgar), MeHg (0.012 mmol/kg, 1/100 of cinnabar) and $HgCl_2$ (0.118 mmol/kg, 1/10 of cinnabar). Mice were dosed daily for six weeks. Animals were closely monitored throughout the entire experiment period. The moribund animals were euthanized to collect tissues. At the end of experiment, animal body weights were recorded, and blood and kidneys were collected for further analysis.

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 a Leica microscope. DP image software was used to capture images.

Blood biochemistry

Blood was collected and allowed to clot at 4°C to separate serum by centrifugation at 3500*g* for 10 min. The concentrations of blood urea nitrogen (BUN) and serum creatinine (CREA) were quantified to evaluate the potential nephrotoxicity with an Automatic Biochemical Analyzer at Zunyi Medical College Hospital.

Determination of As and Hg

A portion of kidney, weighing about 100 mg, was digested in 5 mL 65% nitric acid at 163°C for two hours, and brought to 25 mL with distilled water. Aliquots of 5 mL were incubated for 30 min with 5% sulfourea and ascorbic acid solution, and then As and Hg contents were determined with Atomic Fluorescence Spectrometry (Kechuang Haiguan Instrument Co. Ltd, Beijing, China). These assays were performed by the Guizhou Chemical Analysis Center of Chinese Academia of Sciences.

RNA isolation and realtime polymerase chain reaction analysis

Approximately 50–100 mg of kidney tissue was homogenized in 1 mL TRIzol (Invitrogen, Carlsbad, CA, USA) and total RNA was extracted according to the manufacturer's instructions, followed by purification with RNeasy kits (Qiagen, Valencia, CA, USA). The quality of RNA was determined by the 260/280 ratios. Purified RNA was reverse transcribed with Oligo-dT primers and MuLV reverse transcriptase. The Power SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) was used for realtime reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. The primers were designed by the Primer3 software and are listed in Table 1. The expression of interested genes was first normalized with 18S of the same sample, and the relative transcript levels were calculated setting control as 100%.

Statistical analysis

Data were expressed as mean and standard error. The SPSS 16 software was used for statistical analysis. For comparisons among three or more groups, data were analyzed using a one-way analysis of variance, followed by multiple range Duncan's test. A P value <0.05 was considered statistically significant.

Results

Clinical symptoms

Animal body weights were recorded for the general health. In the beginning of the experiment, the body weights of mice were about 25 ± 2 g. At the end of the experiment, the differences of body weight gain among groups were evident (Figure 1). A significant loss of body weight occurred with $HgCl_2$ and MeHg (31.4, 30.2 versus 38.3 g in control). It should be noted that the doses of $HgCl_2$ and MeHg were only 1/10 and 1/100 of cinnabar, respectively. In the AGNH, cinnabar, realgar, arsenite and arsenate groups, all mice had normal body weight gain. After four weeks of MeHg and $HgCl_2$ administration, the spontaneous

Table 1 Sequences of the primers

Gene	GenBank number	Forward	Reverse
18S	X56974	CGAACGTCTGCCCTATCAACTT	CCGGAATCGAACCCTGATT
MT-1	BC027262	AATGTGCCCAGGGCTGTGT	GCTGGGTTGGTCCGATACTATT
HO-1	M33203	CCTCACTGGCAGGAAATCATC	CCTCGTGGAGACGCTTTACATA
mKC	NM_008176	TGGCTGGGATTCACCTCAAG	GTGGCTATGACTTCGGTTTGG
GST-α4	AK008490	CTATGTTGAGGTGGTCAGGACTGT	CTGTGGTGACACTGCAATTGG
S100a9	BC027635	GGCAAAGGCTGTGGGAAGTA	CCCAGAACAAAGGCCATTGA
E-cadherin	NM_009864	CGATTCAAAGTGGCGACAGA	AGGAAACTGGTCTCCAGCTTGT

activities, grooming and food/water intake of mice were reduced. After five weeks of MeHg administration, some animals had piloerection and could not move normally. Three moribund mice in MeHg group were euthanized to collect blood and kidneys before the end of the experiment.

Blood biochemistry

Two major biomarkers of renal injury, BUN and CREA, were assayed (Figure 2). Figure 2 shows that BUN and CREA were increased after MeHg only (12.2 versus 6.7; 17.8 versus 10.6 mmol/L as compared to control). The levels of BUN and CREA in other groups were not different from controls, indicative of renal injury by MeHg.

Renal pathology

The representative histopathology is shown in Figure 3. In the $HgCl_2$ and MeHg groups, glomeruli were shrunk and the corpuscle space disappeared, and tubular cells were swollen. Cell degeneration with protein cysts in the tubular lumen was evident. Apoptosis (arrows), cell death (arrowhead) and inflammation (big arrows) could be seen. In the arsenite and arsenate groups, glomeruli were shrunk with tubular cells swollen. Foci of tubular cell vacuolation degeneration, and protein cysts in the tubular lumen, and foci of cell death were evident. In the realgar group, mild tubular degeneration and foci of cell death could be seen. In comparison, the AGNH and cinnabar groups showed a largely normal appearance. Glomeruli were intact, tubular cells were slightly swollen and no foci of cell death were found. Considering renal pathology observed in the $HgCl_2$, arsenite and arsenate groups, serum BUN and CREA were not sensitive enough to predict these pathological alterations.

Renal Hg accumulation

A portion of kidney tissue was digested in HNO_3 to determine the accumulation of Hg in the kidney (Figure 4). The

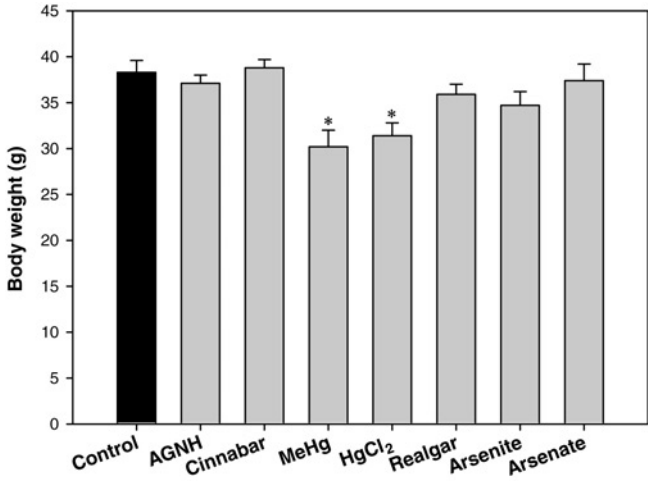

Figure 1 Animal body weights. Mice were administrated orally with AGNH (3 g/kg), cinnabar (0.3 g/kg), realgar (0.3 g/kg), $HgCl_2$ (0.118 mmol/kg, 1/10 of cinnabar), MeHg (0.012 mmol/kg, 1/100 of cinnabar), $NaAsO_2$ (As^{3+} 0.028 mmol/kg, 1/100 of realgar) or Na_2HAsO_4 (As^{5+} 0.056 mmol/kg, 1/50 of realgar), daily for six weeks and body weights were calculated at the end of the experiment. Data are mean $\pm$ SE of six mice. *Significantly different from controls, $P < 0.05$. AGNH, An-Gong-Niu-Huang Wan

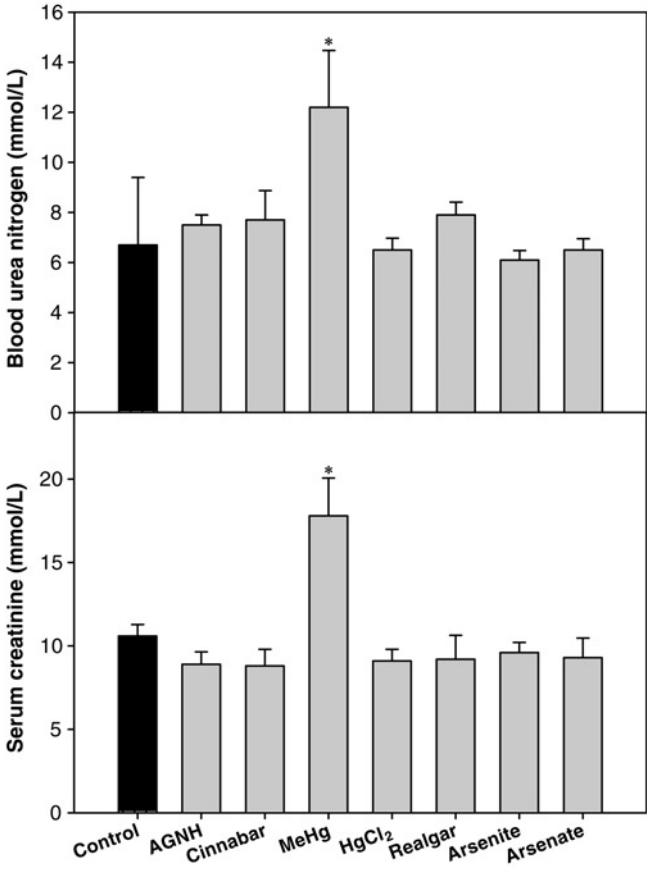

Figure 2 Blood urea nitrogen (top) and creatinine (bottom) levels. Mice were administrated orally with AGNH (3 g/kg), cinnabar (0.3 g/kg), realgar (0.3 g/kg), $HgCl_2$ (0.118 mmol/kg), MeHg (0.012 mmol/kg, 1/100 of cinnabar), $NaAsO_2$ (As^{3+} 0.028 mmol/kg) or Na_2HAsO_4 (As^{5+} 0.056 mmol/kg, 1/50 of realgar), daily for six weeks. Blood was collected at the end of the experiment for analysis. Data are mean $\pm$ SE of six mice. *Significantly different from controls, $P < 0.05$. AGNH, An-Gong-Niu-Huang Wan

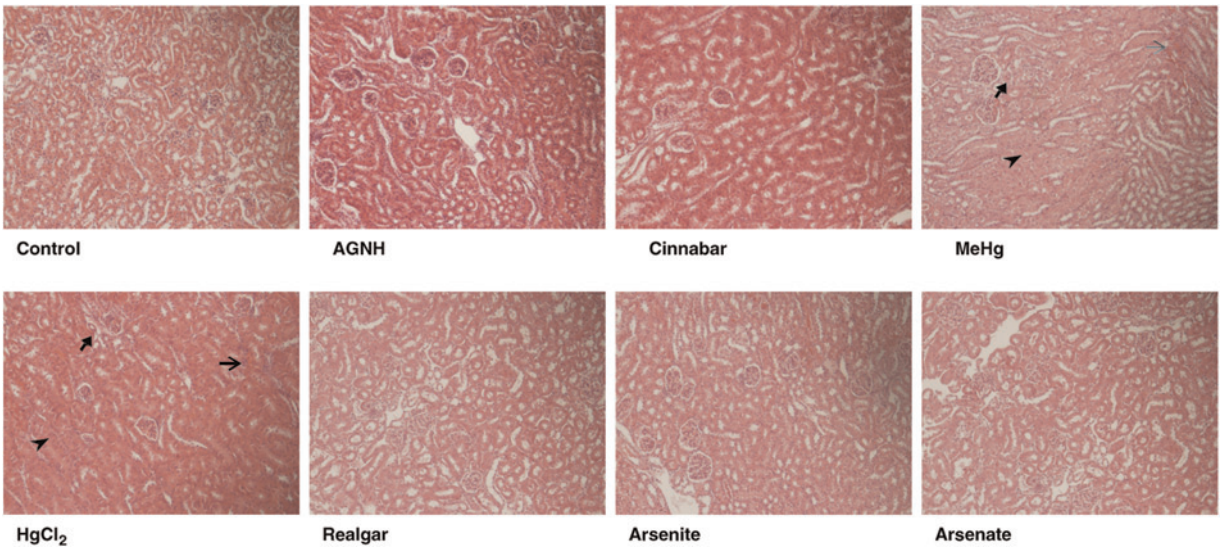

Figure 3 Representative histopathology of the kidney. Mice were administrated orally with AGNH (3 g/kg), cinnabar (0.3 g/kg), realgar (0.3 g/kg), $HgCl_2$ (0.118 mmol/kg), MeHg (0.012 mmol/kg, 1/100 of cinnabar), $NaAsO_2$ (As^{3+} 0.028 mmol/kg) or Na_2HAsO_4 (As^{5+} 0.056 mmol/kg, 1/50 of realgar), daily for six weeks. Tissues were fixed in formalin and stained with hematoxylin and eosin. Arrows point to apoptosis; arrowheads point to cell death and big arrows point to inflammation. Magnitude (×100). AGHN, An-Gong-Niu-Huang Wan. (A color version of this figure is available in the online journal)

accumulation of Hg after MeHg and $HgCl_2$ was increased by 100-(25.56 μg/g) and 400-fold (108.2 μg/g) over control (0.221 μg/g). The Hg accumulation after cinnabar was only doubled (0.56 μg/g), but at much lower levels than the MeHg and $HgCl_2$ groups. Accumulation of Hg after cinnabar in mouse kidneys was comparable to that after chronic cinnabar administration in rats.[15]

Expression of renal toxicity-sensitive genes

Metallothionein-1 (MT-1) is a small, cysteine-rich, metal-binding protein playing an important role in arsenic and mercury detoxification. Induction of MT is widely used as a biomarker for metal-induced toxicity.[16] Heme oxygenase-1 (HO-1) induction is a sensitive index for arsenic-induced stress.[17] In this study, the expression of MT-1 mRNA was increased by arsenite (2×), arsenate

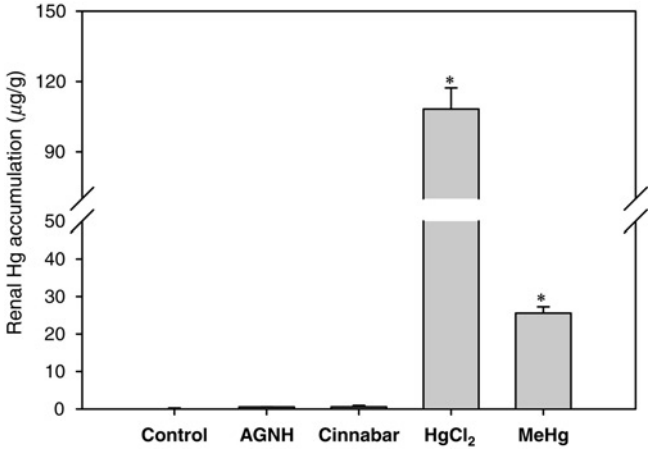

Figure 4 Hg contents in the kidney. Mice were administrated orally with AGNH (3 g/kg), cinnabar (0.3 g/kg), realgar (0.3 g/kg), $HgCl_2$ (0.118 mmol/kg), MeHg (0.012 mmol/kg, 1/100 of cinnabar), $NaAsO_2$ (As^{3+} 0.028 mmol/kg) or Na_2HAsO_4 (As^{5+} 0.056 mmol/kg, 1/50 of realgar), daily for six weeks. Tissues were digested in HNO_3, followed by analysis by atomic fluorescence spectrometry. Data are mean ± SE of six mice and expressed as ng As/g wet tissue. *Significantly different from controls, $P < 0.05$. AGNH, An-Gong-Niu-Huang Wan

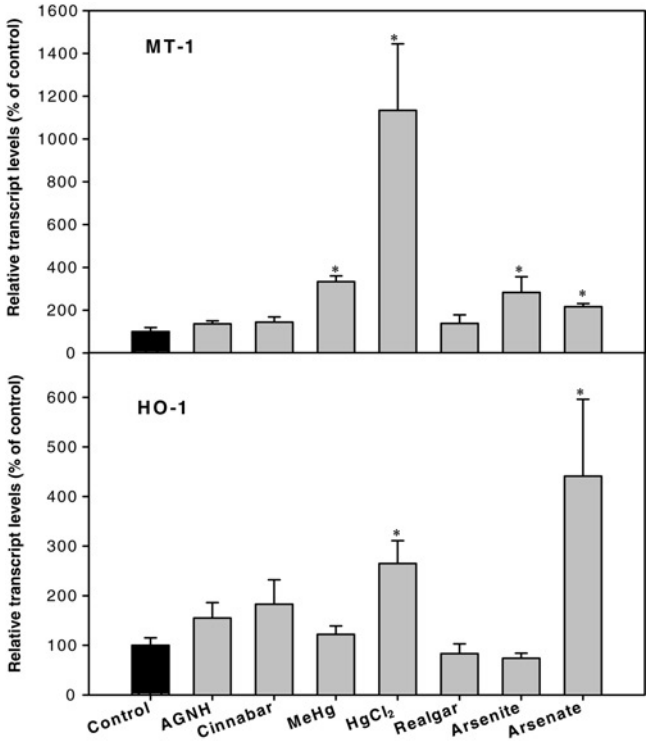

Figure 5 Renal metallothionein-1 (MT-1) (top) and heme oxygenase-1 (HO-1) (bottom) expression. Mice were administrated orally with AGNH (3 g/kg), cinnabar (0.3 g/kg), realgar (0.3 g/kg), $HgCl_2$ (0.118 mmol/kg), MeHg (0.012 mmol/kg, 1/100 of cinnabar), $NaAsO_2$ (As^{3+} 0.028 mmol/kg) or Na_2HAsO_4 (As^{5+} 0.056 mmol/kg, 1/50 of realgar), daily for six weeks. Total RNA was isolated for realtime reverse transcriptase polymerase chain reaction analysis. Data are mean ± SE of six mice. *Significantly different from controls, $P < 0.05$. AGNH, An-Gong-Niu-Huang Wan

(2×), MeHg (3×) and $HgCl_2$ (10×) (Figure 5, top), In contrast, the expression of MT-1 in the AGNH, realgar and cinnabar groups were unchanged. HO-1 expression was increased two-fold by $HgCl_2$ and four-fold by arsenate but was not increased by AGNH, cinnabar and realgar (Figure 5, bottom).

CXC chemokine (mKC) is an important proinflammation mediator in acute and chronic inflammation. Glutathione *S*-transferase (GST) plays an important detoxification role through the consumption of endogenous glutathione by *S*-conjugate formation to prevent metal-induced oxidative stress. The expression of mKC was elevated by MeHg and $HgCl_2$, while it was unchanged in other groups. The expression of GST-α4 was increased by MeHg, $HgCl_2$, arsenite, arsenate and realgar. No significant elevations of GST-4α were seen in the AGNH and cinnabar groups (Figure 6).

E-cadherin and S100 calcium-binding protein A9 (S100a9) play pivotal roles in cellular adhesion and cellular function. In this study, the expression of S100a9 was elevated by MeHg, $HgCl_2$, arsenite and arsenate. Expression of S100a9 was unchanged in the AGNH and cinnabar groups. Expression of E-cadherin, on the other hand, was decreased by $HgCl_2$, arsenite and realgar, but was unchanged by AGNH and cinnabar (Figure 7).

Discussion

This study clearly demonstrated that AGNH, realgar and cinnabar are much less nephrotoxic than MeHg, $HgCl_2$, arsenite and arsenate after chronic oral administrations. Gross toxicity was evident in the MeHg and $HgCl_2$ groups, but not in the realgar, cinnabar and AGNH groups. Histopathology and biochemistry showed severe kidney injury in the MeHg and $HgCl_2$ groups, moderate injury in the arsenite, arsenate and realgar groups, but mild in the cinnabar and AGNH groups. Dramatic renal Hg accumulation occurred after $HgCl_2$ (400 folds) and MeHg (100 folds), but not after AGNH and cinnabar (2.3–2.5 folds) administration. Renal injury-related gene expressions were altered in the MeHg, $HgCl_2$, arsenite or arsenate groups, but were largely unchanged in the AGNH and cinnabar groups. Considering the 100-fold higher doses of realgar, cinnabar and AGNH over arsenite and MeHg used in this study, their nephrotoxic potentials should be much less than MeHg, $HgCl_2$, arsenite and arsenate.

The differential toxicity of arsenicals and mercurials could be due to differences in their toxicokinetics properties.

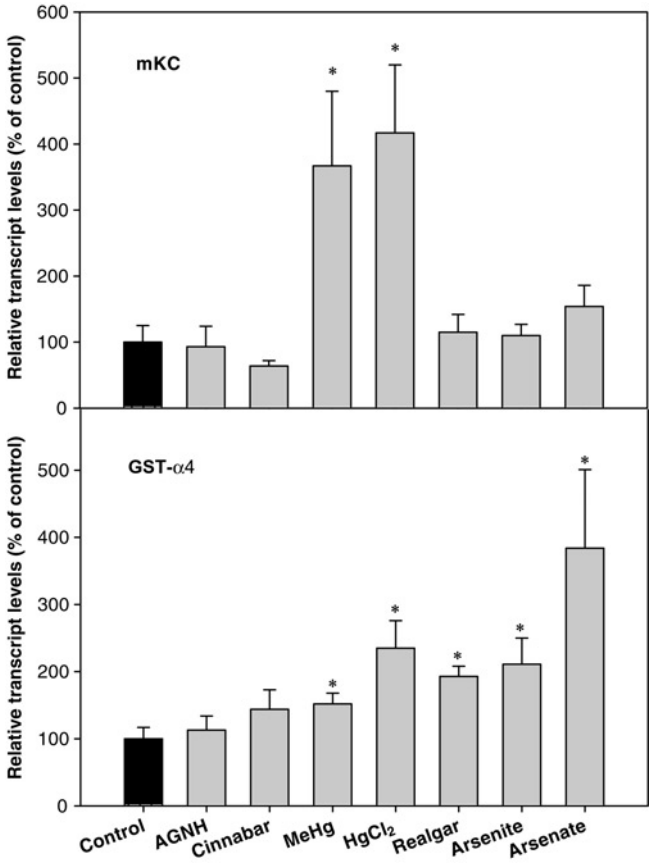

Figure 6 Expression of chemokine (mKC) (top) and glutathione *S*-transferase (GST) (bottom) in the kidney. Mice were administrated orally with AGNH (3 g/kg), cinnabar (0.3 g/kg), realgar (0.3 g/kg), $HgCl_2$ (0.118 mmol/kg), MeHg (0.012 mmol/kg, 1/100 of cinnabar), $NaAsO_2$ (As^{3+} 0.028 mmol/kg) or Na_2HAsO_4 (As^{5+} 0.056 mmol/kg, 1/50 of realgar), daily for six weeks. Total RNA was isolated for realtime reverse transcriptase polymerase chain reaction analysis. Data are mean ± SE of six mice. *Significantly different from controls, $P < 0.05$. AGNH, An-Gong-Niu-Huang Wan

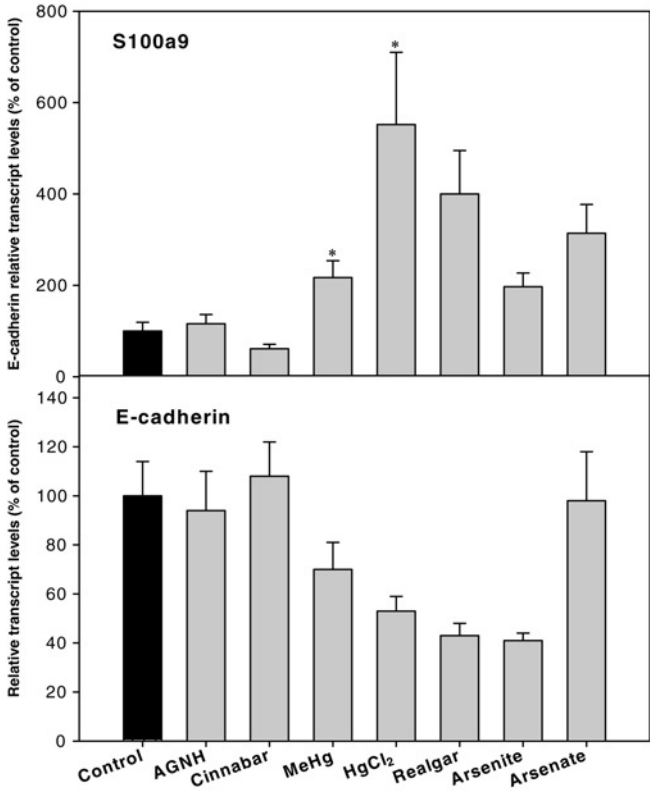

Figure 7 Expression of S100a9 (top) and E-cadherin (bottom) in the kidney. Mice were administrated orally with AGNH (3 g/kg), cinnabar (0.3 g/kg), realgar (0.3 g/kg), $HgCl_2$ (0.118 mmol/kg), MeHg (0.012 mmol/kg, 1/100 of cinnabar), $NaAsO_2$ (As^{3+} 0.028 mmol/kg) or Na_2HAsO_4 (As^{5+} 0.056 mmol/kg, 1/50 of realgar), daily for six weeks. Total RNA was isolated for realtime reverse transcriptase polymerase chain reaction analysis. Data are mean ± SE of six mice. *Significantly different from controls, $P < 0.05$. AGNH, An-Gong-Niu-Huang Wan

Realgar has a low solubility in water, and only 4% is bioavailable in physiological gastric juice or intestinal fluid.[18] Oral administration of realgar in rats (150 mg/kg, daily for 5 weeks) showed that only a small portion of arsenic was absorbed and reached the blood, lung, spleen or liver.[19] Trivalent arsenic is highly water-soluble and well absorbed after an oral dose. Thus, the oral LD_{50} in rodents is 30–45 mg for arsenite, but 3.2 g/kg for realgar.[2] The solubility and bioavailability of cinnabar are quite low as well. The water solubility of $HgCl_2$ is 30–70 g/L, but cinnabar is less than 0.001 g/L at 20°C.[20] Bioavailability of cinnabar is at least 30–60 folds less than $HgCl_2$.[21] Absorption of cinnabar (<0.2%) from the gastrointestinal tract is much less than $HgCl_2$ (7–15%). In our recent acute study, kidney Hg accumulation from $HgCl_2$ was 100 times more than that from cinnabar.[22] Bioavailability is a critical determinant of toxicity of metal compounds. We have attempted to quantify kidney As content, but the As content was low or even under the detection limit because of rapid As elimination during chronic exposures.[23,24] It is the amount of toxicants to the target organ rather than the amount ingested that makes a poison.[25] For example, neurotoxic effects produced by cinnabar were estimated to be about 1/1000 of those induced by MeHg.[26] Understanding better the toxicokinetics of realgar and cinnabar is very important for appropriate safety assessment of cinnabar- and realgar-containing traditional medicines.

Oxidative stress has been proposed as an important mechanism involved in metal toxicity. MT is a small, cysteine-rich, metal-binding protein playing an important role in metal toxicity.[16] Both arsenic and mercury are efficient MT inducers,[16,27] and MT-null mice were susceptible to chronic arsenic-induced hepatotoxicity and nephrotoxicity,[28] as well as to $HgCl_2$-induced renal injury.[29] MT induction is a sensitive biomarker for metal-induced stress. In this study, the expression of MT-1 was increased by arsenite, arsenate, MeHg and $HgCl_2$, but was unaltered in the realgar, cinnabar and AGNH groups. Similarly, induction of HO-1 is a hallmark of As-induced oxidative stress.[17] In this study, marked induction of HO-1 was observed in both the $HgCl_2$ and arsenate groups but HO-1 expression was unaltered in the realgar, cinnabar and AGNH groups. Indeed, arsenite could cause more oxicative DNA damage than realgar.[30]

Inflammation plays an important role in metal-reduced toxicity. Chemokine (mKC) is an important proinflammation mediator in acute and chronic renal disease.[31,32] In this study, mKC was increased after MeHg and $HgCl_2$ treatment, indicative of inflammatory response, but it was unchanged in other groups. GST increase can be a sensitive biomarker for kidney injury.[33,34] In this study, the expression of GST-α4 was increased by MeHg, $HgCl_2$, arsenite, arsenate and realgar. No significant elevation of GST-4α was seen in the AGNH and cinnabar groups, consistent with their nephrotoxicity potentials.

S100a9 is a calcium-binding protein highly expressed in neutrophil and monocyte cytosol, and its overexpression is a sign of dysfunction of cellular adhesion.[35] In this study, S100a9 was increased by arsenite, arsenate, MeHg and $HgCl_2$, and was unaltered after cinnabar and AGNH, consistent with histopathology. E-cadherin is essential for maintaining the epithelial polarity and barrier integrity that is necessary for the normal absorption/excretion of fluid and solutes in the kidney and is an important early target for a variety of nephrotoxic substances including metals, such as Hg.[36] Our study showed that the expression of E-cadherin was decreased not only by $HgCl_2$ but also by arsenite and realgar. E-cadherin was unchanged by AGNH, cinnabar, MeHg and arsenate.

It should also be noted that although the toxicity of realgar, cinnabar and AGNH was much less than MeHg, $HgCl_2$, arsenite and arsenate, the long-term or high-dose administration of cinnabar caused renal morphology changes.[15] Long-term administration of cinnabar and realgar also induce mild injury in the rat liver and kidney.[37,38] 'Dose makes a poison'; therefore, caution should be taken to avoid high doses and the long-term use of realgar- and cinnabar-containing traditional medicines.

Summary

This work clearly showed that toxicity potentials of realgar, cinnabar and AGNH were much less than the common arsenicals (arsenite and arsenate) and mercurials (MeHg and $HgCl_2$). These differences appeared to be due to both toxicokinetics, with less metal accumulation in target tissues and toxicodynamics, as evident by biomarker gene expression. Thus, the chemical forms of realgar and cinnabar should be taken into consideration for safety evaluation and the use of total As and Hg contents to regulate realgar- and cinnabar-containing traditional medicines lacks sound scientific basis.

Author contributions: Y-FL was responsible for critical contributions on experiment design, obtaining and processing data, data interpretation and manuscript writing. QW, J-WY and J-ZS were responsible for important contributions on experiment performance and data interpretation. JL was responsible for the experiment hypothesis, design and conduction, as well as overall direction of the entire project. J-SS was involved in experiment design and data discussion.

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