Perspectives in Pharmacology

Mineral Arsenicals in Traditional Medicines: Orpiment, Realgar, and Arsenolite

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ABSTRACT

Mineral arsencals have long been used in traditional medicines for various diseases, yet arsenic can be highly toxic and carcinogenic. Arsenic in traditional medicines typically comes from deliberate addition for therapeutic purposes, mainly in the form of mineral arsencals, including orpiment (As₂S₃), realgar (As₄S₄), and arsenolite (contains arsenic trioxide, As₂O₃). Inorganic arsenic is now accepted in Western medicine as a first line chemotherapeutic agent against certain hematopoietic cancers. This perspective analyzes the pharmacology and toxicology of these arsencals used in traditional medicines. Orpiment and realgar are less soluble and poorly absorbed from the gastrointestinal tract, whereas the bioavailability of arsenic trioxide is similar to inorganic arsenic salts such as sodium arsencite. Pharmacological studies show that arsenic trioxide and realgar are effective against certain malignancies. Orpiment and realgar are used externally for various skin diseases. Realgar is frequently included as an ingredient in oral traditional remedies for its antipyretic, anti-inflammatory, antilucre, anti-convulsive, and anti-schistosomiasis actions, but the pharmacological basis for this inclusion still remains to be fully justified. Toxicological studies show that cardiovascular toxicity is the major concern for arsenic trioxide and that the gastrointestinal and dermal adverse effects may occur after prolonged use of mineral arsencals. Little is known regarding the possible secondary cancers resulting from the long-term use of any of these arsencals. Similar to the safety evaluation of seafood arsencals, total arsenic content alone appears to be insufficient for mineral arsenical safety evaluation. Arsenic speciation, bioavailability, and toxicity/benefit should be considered in evaluation of mineral arsencal-containing traditional medicines.

Traditional medicines, mainly Chinese medicines and Indian Ayurvedic medicines, are becoming more and more popular as alternative and supplementary remedies over recent years (Kumar et al., 2006; Efferth et al., 2007). Toxic metals or metalloids, such as lead, mercury, and arsenic, are frequently found in traditional medicines, raising justifiably escalating public concerns (Ernst, 2002; Cooper et al., 2007). Indeed, at least for arsencals, many traditional medicines call for intentional addition of mineral arsencals based on their presumed or defined therapeutic properties (Ernst, 2002; Miller et al., 2002; Evens et al., 2004; Chinese Pharmacopeia Committee, 2005; Efferth et al., 2007). However, arsenic is a known human carcinogen producing cancers of the skin, lung, urinary bladder, liver, kidney, and possibly other sites (National Research Council, 1999; IARC, 2004) and has many other profound toxic effects following short-term or long-term exposure (National Research Council, 1999; Agency for Toxic Substances and Disease Registry, 2005; Liu et al., 2007). The general perception is that intentional addition of a known carcinogen to any medicine is a preposterous risk. The conundrum is that arsencals have a long and remarkable history of pharmacology utility. Nonetheless, arsenic used in traditional medicines alarms the public, and this perspective will first assess the available database on mineral arsencals in traditional medicines and then attempt to analyze their risk in light of their potential benefit.

Use of Mineral Arsenicals in Traditional Medicines

Arsenic has been used as a poison and as a therapeutic since ancient times (Miller et al., 2002; Agency for Toxic...
Substances and Disease Registry, 2005; Liu et al., 2007). In ancient Chinese medicines, the use of arsenic can be traced back to 200 B.C. in Shen Nong Ban Cao Jing, the first traditional Chinese medicine book. Using a poison to attack another poison or to fight against malignant diseases is a common concept in traditional Chinese medicines (Evens et al., 2004; Chinese Pharmacopeia Committee, 2005). The use of mineral elixir made from the “essence of the five planets,” including arsenic-containing minerals, was thought to give humans perpetual life in Indian Ayurvedic medicines (Kumar et al., 2006). Table 1 lists properties of the three major arsenic-containing minerals used in traditional medicines. These arsenicals include orpiment, which is also called yellow arsenic, Arsenikon (Greek) or Cihuang (China), and contains As$_2$S$_3$. Another is realgar, which is also called red arsenic due to a deep red color, or Xionghuang (China), and contains >90% arsenic disulfide (As$_2$S$_2$) or As$_2$S$_3$. Arsenolite, which is the third common mineral arsenical called white arsenic, contains largely As$_2$O$_3$. Physicians prescribed arsenicals for both external and internal use throughout the 19th century (Miller et al., 2002; Evens et al., 2004). Arsenic and arsenic salts were key ingredients in antiseptics, antispasmodics, hematinics, sedatives, ulcer, and cancer cures. Arsenical preparations, such as Fowler solution (1% potassium arsenite), were used by many physicians in the treatment of malignant diseases, such as leukemia, Hodgkin’s disease, pernicious anemia, and nonmalignant diseases, such as psoriasis, pemphigus, eczema, and asthma for centuries (Miller et al., 2002; Evens et al., 2004). Arsenic was the standard therapy for syphilis for nearly 40 years before it was replaced by penicillin. Approximately 60 different arsenic preparations have been developed and used during the lengthy pharmacological history of arsenic until their uses were gradually replaced by more effective and less toxic modern agents (Miller et al., 2002; Evens et al., 2004; Effreth et al., 2007). Today, hundreds of traditional Chinese medicines still use orpiment, realgar, or arsenolite, and realgar alone is included in 22 oral remedies based on Chinese Pharmacopeia Committee (2005). In Indian Ayurvedic medicines, realgar is also a major component in bhasmas (Mitra et al., 2002; Kumar et al., 2006). Arsenic trioxide is now becoming a very promising chemotherapeutic agent in Western medicine to treat acute promyelocytic leukemia (APL) and possibly other malignantancies (Miller et al., 2002; Evens et al., 2004; Hede, 2007).

**Arsenic Species and Their Short-Term Toxicity**

Arsenic exists in the trivalent and pentavalent forms and is widely distributed in nature. The most common toxic inorganic arsenic compounds are sodium arsenate (As$_5^{+}$) and sodium arsenite (As$_3^{+}$). In the body, arsenate can be reduced to arsenite, followed by conjugative methylation reaction to form monomethylarsononic acid (MMA), then dimethylarsinic acid (DMA), and finally trimethylarsonic acid (TMA), with these methylated species found in urine (Fig. 1A) (Liu et al., 2007). Arsenic toxicity is highly dependent on the chemical form, and where known, the acute oral LD$_{50}$ values in rodents are also included under each arsenic compound in Fig. 1. In general, sodium arsenate (LD$_{50}$ 112–175 mg/kg) is four to five times less acutely toxic than sodium arsenite (LD$_{50}$ 15–44 mg/kg), and the pentavalent organic arsenuclals, MMA (LD$_{50}$ 960 mg/kg), DMA (LD$_{50}$ 650 mg/kg), and TMA (LD$_{50}$ 16.7 mg/kg) are 40 to 100 times less acutely toxic than arsenite (Kreppel et al., 1993; Agency for Toxic Substances and Disease Registry, 2005). Arsenicals in seafood mainly exist as organic forms (Fig. 1B), such as arsenobetaine (LD$_{50}$ 10 g/kg), arsenosugar (not available), and arsenocholine (LD$_{50}$ 6.5 g/kg) (Borak and Hosgood, 2007), with acute oral LD$_{50}$ values 100 to 500-fold above arsenite or arsenate. In traditional medicines, natural arsenic-containing minerals are used as drugs, such as orpiment, realgar, and arsenolite (Fig. 1C). The oral LD$_{50}$ for arsenic trioxide (i.e., arsenolite) in mice is 33 to 39 mg/kg (Carter et al., 2003), similar to sodium arsenite, but the LD$_{50}$ for realgar is 3.2 g/kg, a difference of 100-fold compared with sodium arsenite (Zhang et al., 2004). The oral LD$_{50}$ for orpiment is not available, possibly because orpiment is mainly for external use (Chinese Pharmacopeia Committee, 2005). The wide range of LD$_{50}$ values among different arsenicals clearly indicates that mineral arsenical toxicity is highly dependent on the chemical form.

**Bioavailability of Orpiment, Realgar, and Arsenolite/Arsenic Trioxide**

It is generally assumed that the severity of poisoning is related to the total amount of poison ingested, and assessment of health risk associated with arsenic exposure from human ingestion of traditional medicines has typically taken this tactic (Ernst, 2002; Cooper et al., 2007). However, in many cases, a significant portion of some forms of mineral arsenicals are poorly absorbed into the body and would be unavailable to cause systemic damage. The disposition of these arsenicals in the body depends on various key factors, including solubility, absorption, distribution, and excretion. Table 2 lists the available data on disposition of these mineral arsenicals.

Orpiment has low solubility in water. Orpiment dissolution is kinetically slow and under anaerobic conditions; an increase in pH increases orpiment dissolution rate (Floroiu and Davis, 2004). For instance, in aqueous solution, more arsenic from orpiment is dissolved at pH 7 than at pH 4 (Marafante and Vaher, 1987). When orpiment is incubated in a cell culture media, 3% arsenic is released, which is actually de-
creased in the presence of pulmonary macrophages (Lantz et al., 1995). Macrophages engulf particles into phagosomes, which have an acidic milieu (Floroiu and Davis, 2004). Orally administrated orpiment is poorly absorbed, and over 82% is found in feces within 3 days, representing an unabsorbed portion of the dose compared to only 12% of an oral dose of sodium arsenate. Urinary arsenic metabolites from oral orpiment exposure are mainly DMA, suggesting that the bio-transformation of absorbed orpiment arsenicals occurs in the body (Marafante and Vahter, 1987).

Realgar in Niuhuang Jiedu Pian, a common preparation for a common cold, has a low solubility in water, and only 4% is bioavailable in physiological gastric juice or intestinal fluid (Koch et al., 2007). The average total arsenic concentration in a Niuhuang Jiedu Pian is approximately 71100 ppm, corresponding to 28 mg of arsenic per pill, of which only 1 mg of arsenic finds its way into the blood stream, and 40% of this absorbed arsenic (0.4 mg) is excreted in urine (Koch et al., 2007). Realgar exposure results in various arsenical metabolites in the urine, including MMA, DMA, arsenobetaine, and an unknown metabolite, the level of which peaked at approximately 14 h after ingestion (Koch et al., 2007). In healthy volunteers, <1% of total administered arsenic was found in the urine after repeated doses of Niuhuang Jiedu Pian (three tablets, twice a day) during a 7-day period (Tang and Wang, 2005). 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 (45 mg/ml), lung (5.4 mg/g), spleen (5.2 mg/g), or liver (2.9 mg/g) (Tang and Wang, 2005). To overcome the low solubility and poor bioavailability, realgar nanoparticles have been prepared by cryogrinding with polyvinylpyrrolidone and SDS, and arsenic solubility can greatly be increased compared to crude realgar powder (Wu and Ho, 2006). Realgar nanoparticles show remarkable increases in bioavailability both in vitro and in vivo. For example, urinary recovery of arsenic in rats after a single oral administration of realgar nanoparticles (50 mg/kg p.o.) was increased to 70% of the dose compared to 25% when realgar was given in crude powder (Wu and Ho, 2006).

Arsenic trioxide, purified from mineral arsenolite, is highly water-soluble and well absorbed after oral dose. Thus, the oral LD50 in mice for arsenic trioxide is very close to that of sodium arsenite (Carter et al., 2003). Pharmacokinetic studies in humans show that after arsenic trioxide infusion (10 mg/day i.v.) for ~90 days for cancer chemotherapy, blood arsenite levels reached steady state of 5.5 to 7.3 μM (Shen et al., 1997). In another study, patients received repeated administrations of arsenic trioxide at similar doses and duration, plasma concentration of arsenic reached a steady state after 4 weeks of treatment, and 60% arsenic dose was excreted in urine in the forms of arsenite (14%), arsenate (7%), MMA (19%), and DMA (21%) (Fujisawa et al., 2007). Compared to intravenous administration, orally given arsenic trioxide can achieve similar mean plasma levels (Kumana et

Table 2

<table>
<thead>
<tr>
<th>Arsenicals</th>
<th>Bioavailability</th>
<th>System</th>
<th>Major Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orpiment</td>
<td>Low</td>
<td>In vitro</td>
<td>Dissolution increase with pH increase</td>
<td>Lantz et al., 1995; Floroiu et al., 2004</td>
</tr>
<tr>
<td>Reaglar</td>
<td>4%</td>
<td>Human</td>
<td>MMA, DMA in urine</td>
<td>Marafente and Vahter, 1987</td>
</tr>
<tr>
<td>Reaglar Nanoparticles</td>
<td>Low</td>
<td>Rat</td>
<td>Blood &gt; lung, heart &gt; spleen &gt; liver, kidney</td>
<td>Wang et al., 2003</td>
</tr>
<tr>
<td>Arsenic trioxide</td>
<td>High</td>
<td>Rabbit</td>
<td>As3+ in blood, MMA, DMA in liver and lung</td>
<td>Wu and Ho, 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human</td>
<td>60% i.v. dose recovery in urine</td>
<td>Fujisawa et al., 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human</td>
<td>Oral equals to i.v. bioavailability</td>
<td>Kumana et al., 2002</td>
</tr>
</tbody>
</table>

Fig. 1. Acute oral toxicity (LD50) of arsenicals in rodents. A, common inorganic arsenicals and their organic arsenical metabolites. B, arsenic species in seafood. C, mineral arsenicals. N/A, LD50 data are not available.
the counter preparation *Niuhuang Jiedu Pain* contains 6.4% realgar, and the bioavailability of arsenic released from this preparation is very low (Koch et al., 2007). The therapeutic uses of these preparations range widely, for instance, for common colds, toothache, and tonsillitis, asthma, abdominal pains, spasms, sedation, ulcers, heat stroke, coma, and delirium. Few pharmacologic studies on these preparations are found in the English literature. The interactions of realgar with other herbs or minerals, such as cinnabar (HgS), in many cases are unknown. In this perspective, only the anti-cancer effects of realgar are briefly discussed. To enhance therapeutic efficacy and reduce adverse effects, physicians of traditional Chinese medicine prescribe the combination formulae of plant species/minerals based on clinical experience, and thousands of such formulae have been recorded (Wang et al., 2008). For example, *Auei Huapi Gao*, a preparation containing 4% realgar, appears effective against “lumps” or various malignancies in traditional therapies. Since the 1960s, realgar-containing preparations, such as *Fufan Qingdai Pian*, *Kebai Dan*, *Manli Pian*, etc., have been successfully used in the treatment of certain types of acute and chronic leukemia (Chen et al., 2000). When the realgar amount is doubled, as in *Fufan Qingdai Pian*, a better antitumor response is achieved (Chen et al., 2000). Realgar is less toxic compared to arsenic trioxide and is now used alone or in combination for hematologic malignancies (Lu et al., 2002; Shen et al., 2004). Recently, Realgar-*Indigo naturalis* formulae have been shown to be very effective against promyelocytic leukemia (Wang et al., 2008). Realgar acts as the principal component of the formula, whereas other plant active ingredients (such as indirubin and trichinone IIA) serve as adjuvant ingredients in inducing acute promyelocytic leukemia cell differentiation and the degradation/ubiquitination of promyelocytic leukemia-retinoic acid receptor-α oncoprotein, in enhancing G_{1}/G_{0} arrest in APL cells through hitting multiple targets, and in intensifying aquaglyceroporin-9 expression and thus facilitating transportation of realgar into APL cells (Wang et al., 2008).

Arsenolite is traditionally used for removing lump or “scrofula” and is included in *Ailin Yihao* as a modification of Chinese Pharmacopeia Committee (2005) in the treatment of acute promyelocytic leukemia (Shen et al., 1997; Chen et al., 2000). Arsenic trioxide is an example of how an active ingre-
Toxicology studies of orpiment, realgar, and arsenolite/arsenic trioxide

Arsenicals have been known as Poisons of the King since ancient times, and it has a variety of short-term and long-term toxic effects, such as skin lesions, vascular toxicity, respiratory, renal, and liver toxicity, most importantly, the carcinogenic potential (IARC, 2004; Agency for Toxic Substances and Disease Registry, 2005). The wide range of LD50 among mineral arsenicals (Fig. 1) points toward the need to discuss the toxicology of mineral arsenicals individually (Table 4).

Intraperitoneal administration of orpiment was negative in mouse bone marrow cell micronucleus assay, despite the resultant of very high blood arsenic levels (900 ng/ml) (Tinwell et al., 1991). Intratracheal administration of orpiment (3.75 mg/kg, once a week for 15 weeks) in hamsters is also effective against solid tumor cells (Maeda et al., 2004). Arsenic trioxide is also effective against metastatic cervical cancers (Yu et al., 2007). New studies on chemotherapy with arsenic trioxide are underway (Hede, 2007).

In traditional medicine-based therapy, patient treatment commences without any experimental phase in the laboratory. The Western concept of “from bench to bedside” does not fit in the clinical practice of traditional remedies (Efferth et al., 2007). Nonetheless, the pharmacological basis for mineral arsenical inclusion in traditional medicine still remains to be fully justified.

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Arsenic trioxide is highly toxic compared to orpiment and realgar. Short-term toxicity of arsenic trioxide is the major concern in the use of this agent to against malignancies, and at least three sudden deaths have been reported (Westervelt et al., 2001). Prompt chelation treatment is beneficial for short-term arsenic trioxide intoxication; for example, there was a potentially lethal case in which a patient ingested with 9000 mg of arsenic trioxide was rescued by prompt emergency care, forced diuresis, and chelation therapy with 2,3-dimercaptopropanol and meso-2,3-dimercaptopropanol (Vantroyen et al., 2004). The clinical doses of arsenic trioxide (5–10 mg i.v.) could induce cardiac injury, such as QT prolongation, arrhythmias, and, in extreme cases, cardiac arrest (Westervelt et al., 2001; Evens et al., 2004; Chou and Dang, 2005). Other adverse effects include skin lesions, gastrointestinal symptoms (Miller et al., 2002; Chou and Dang, 2005), neuropathy, and liver dysfunction are reported with long-term arsenic trioxide use (Miller et al., 2002; Evens et al., 2004; Chou and Dang, 2005) and are generally tolerable and reversible. In a long-term study in rabbits, arsenic trioxide at a dose of 0.2 mg/kg i.v. for 30 days produced cardiac injury, with alterations in cardiac function. These adverse effects are reversible after the termination of arsenic trioxide treatment (Wu et al., 2003). Possible secondary cancers have not been reported in patients receiving arsenic trioxide (Miller et al., 2002; Evens et al., 2004; Chou and Dang, 2005). However, arsenic-induced cancers may have a long latent period, and

**TABLE 4**

Toxicology studies of orpiment, realgar, and arsenolite/arsenic trioxide

<table>
<thead>
<tr>
<th>Arsenicals</th>
<th>Short-Term Toxicity</th>
<th>Long-Term Toxicity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orpiment</td>
<td>N/A</td>
<td>Negative in micronucleus assay</td>
<td>High as levels in blood</td>
</tr>
<tr>
<td>Reaglar</td>
<td>N/A</td>
<td>Negative in lung tumor formation</td>
<td>Adverse effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cutaneous manifestations</td>
<td>Tolerable</td>
</tr>
<tr>
<td>Arsenic trioxide</td>
<td>Sudden death, poisoning</td>
<td>Prolonged QT, dose-dependent</td>
<td>Long-term exposure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fatty liver, but no liver fibrosis</td>
<td>Chelation effective</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cardiac effects and GI effects</td>
<td>Tolerable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skin and GI effects</td>
<td>No secondary cancer reports</td>
</tr>
</tbody>
</table>

N/A, LD50 data are not available.
the longer time monitoring is needed to verify the carcinogenicity of arsenic trioxide or realgar used in traditional remedies.

“The dose makes a poison”. In the evaluation of the toxic effects of mineral arsenicals, dose and duration of administration should be critically considered. Although mineral arsenicals in traditional medicines are beneficial and even curative of various diseases, it should be kept in mind that “the right dose differentiates a remedy from a poison”. Another important consideration is to balance the benefit and risk. Arsenic trioxide is highly toxic, but to save from malignancies, the use of a poison like arsenic trioxide may be justified.

Summary

This perspective discussed mineral arsenicals used in traditional medicines. Orpiment and realgar have quite different chemical features and solubility from arsenolite/arsenic trioxide. The bioavailability of orpiment and realgar are low; however, arsenolite/arsenic oxide is high. Pharmacologic data indicate that the use of orpiment and realgar in traditional medicines may be desired in some cases; however, the therapeutic basis in most instances remains to be fully justified. Arsenolite/arsenic trioxide has been a major breakthrough as a cure for a subset of human leukemias, and its use as a mineral arsenical in traditional medicines prompted this finding. Cardiovascular toxicity is the major concern for arsenic trioxide, and realgar is much less acutely toxic than arsenic trioxide. Little is known about possible secondary cancers resulting from the long-term use of any of these arsenicals. Similar to the safety evaluation of seafood arsenic, total arsenic content alone is insufficient for safety evaluation of mineral arsenical-containing traditional medicines, and arsenic speciation, bioavailability, and toxicity/benefit should be all considered in any such evaluation.

Acknowledgments

We thank Drs. Wei Qu, Yang Sun, and Larry Keefer for critical review of this perspective.

References