Clinical Manifestations and Arsenic Methylation after a Rare Subacute Arsenic Poisoning Accident

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One hundred and four workers ingested excessive levels of arsenic in an accident caused by leakage of pipeline in a copper-smelting factory. Clinical examinations were performed by physicians in a local hospital. Excreted urinary arsenic species were determined by cold trap hydride generation atomic absorption spectrometry. In the initial toxic phase, gastrointestinal symptoms were predominant (83 people, 79.8%). Most patients showed leucopenia (72 people, 69.2%), and increased serum alanine aminotransferase (84 people, 80.8%) and aspartate aminotransferase (58 people, 55.8%). Thirty-five patients (33.6%) had elevated red blood cells in urine. After 17 days of admission, many subjects (45 people, 43.3%) developed peripheral neuropathy and 25 of these 45 patients (24.0%) showed a decrease in motor and sensory nerve conduction velocity. In the comparison of urinary arsenic metabolites among subacute arsenic-poisoned, chronic high arsenic–exposed and control subjects, we found that subacute arsenic-poisoned patients had significantly elevated proportions of urinary inorganic arsenic (iAs) and methylarsonic acid (MMA) but reduced proportion of urinary dimethylarsinic acid (DMA) compared with chronic high arsenic–exposed and control subjects. Chronic exposed subjects excreted higher proportions of iAs and MMA but lower proportions of DMA in urine compared with control subjects. These results suggest that gastrointestinal symptoms, leucopenia, and hepatic and urinary injury are predominant in the initial phase of subacute arsenic poisoning. Peripheral neuropathy is the most frequent manifestation after the initial phase. The biomethylation of arsenic decreases in a dose rate–dependent manner.

Key Words: subacute arsenic poisoning; arsenic methylation; DMPS; urinary arsenic species

Arsenic, a metalloid, has a long history of use in human civilization. The use of arsenical therapeutics dates back to the times of ancient Rome, when Galen and Hippocrates used them to treat ulcers (Graeme and Pollack, 1998; Seavolt et al., 2002). Arsenic trioxide, realgar, and orpiment are all arsenic compounds as well as remedies in traditional Chinese medicines as recorded in Compendium of Materia Medica. Arsenic trioxide is now used as a very effective cancer chemotherapeutic against certain hematopoietic tumors (Berenson and Yeh, 2006). Today, arsenic compounds are still widely used in industry and agriculture. However, arsenic has been identified as a human carcinogen by the International Agency for Research on Cancer (IARC, 2004). Arsenic exposure can result in both chronic and acute toxicity in humans. Chronic arsenic poisoning is a global health problem affecting millions of people, especially in India, Bangladesh, and China (Ahsan et al., 2000; Mazumder et al., 1998; Sun, 2004). The main cause of the widespread chronic arsicosis is the consumption of underground drinking water naturally contaminated by arsenic. Arsenic contamination of drinking water may also result from mining and other industrial processes. Acute arsenic poisoning is relatively less common but has been documented after accidental ingestion of insecticides or pesticides, and attempted suicides or murders with arsenicals (Lech and Trela, 2005; Ratnaike, 2003).

In contrast to chronic poisonings and acute arsenic overdoses, subacute arsenic poisoning only happens rarely, especially in a large population. This manuscript describes the impact of an accidental subacute exposure to arsenic on 104 patients caused by the waste leakage of pipelines in a copper-smelting factory, mainly as a contribution to the understanding of the clinical features and therapeutics of subacute arsenic poisoning. In addition, relative occurrence of urinary arsenic metabolites was quantified to shed light on arsenic methylation capacity in the subacute arsenic-poisoned patients for the first time. Most mammals metabolize inorganic arsenic (iAs) via methylation to methylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Aposhian et al., 2004). There seems to be variation in the susceptibility to arsenic toxicity. It was suggested that this might be related to variation in arsenic biotransformation in chronic arsenic poisoning (Tseng, 2007). The mechanism for arsenic poisoning is not well understood. Arsenic methylation may be the key to understand the toxicity of arsenic in vivo.

MATERIALS AND METHODS

Background of the poisoning event. From the second to the fifth of December 2004, 26 people from 12 families around a copper-smelting factory...
in Fuxin, China presented at a local hospital with malaise, nausea, vomiting, diarrhea and abdominal pain. The livestock (chickens, ducks) and pets (cats) kept by these families died mysteriously. At the same time, many workers in the factory began showing similar symptoms and went to hospital together, complaining of food poisoning. Expert consultation and field investigation were immediately organized at the beginning of the event. On 5 December, the patients were diagnosed with subacute arsenic poisoning, which was caused by the ingestion of extremely high dose of arsenic in a short term.

This accidental poisoning was caused by the leakage of arsenic-containing waste from the drain pipe in the copper-smelting factory. The leakage was supposed to occur on 28 November 2004. A well which supplied drinking water to the factory and 12 families around was located on the factory grounds, 25 m away from the drain pipe. Apparently soon thereafter the well became polluted by the waste. The local Center for Disease Control and Prevention and our laboratory tested the well water for arsenic. The concentration of arsenic was 48.5 ± 4.3 μg/L, 970 times of the maximum allowable concentration for arsenic in drinking water (0.05 μg/ml) in China. All the workers lived nearby the factory and had breakfast and dinner at home. They had pure water for drinking in the factory. The only source of arsenic exposure for this group would be consumption of lunch cooked with the polluted well water during the work day. The workers presented with symptoms of subacute arsenic poisoning after consuming such lunch for 5–8 continuous days.

A total of 268 people, aged 1.5–70 years old, were admitted to two hospitals in Fuxin city. Most of them had nausea, vomiting, diarrhea and abdominal pain. Twenty-six people from 12 families around the copper-smelting factory and 104 workers of the factory presented with the poisoning related symptoms earlier and more seriously than the rest. They were admitted to the Centre Hospital of Fuxin before 5 December. The rest 138 people who complained of the poisoning symptoms later were admitted to another hospital after 5 December. Some victims of the 138 people, however, did not show elevated levels of urinary arsenic. Severe psychosomatic factors cannot be excluded. Here, we reported on 104 workers admitted in the Central Hospital of Fuxin before 5 December. The members of the 12 families who also consumed the water from the arsenic-contaminated well were not included in our study.

**Clinical examinations.** Clinical examinations were done by doctors in the Central Hospital of Fuxin. All 104 people, aged 18–65 years old, including 94 males (90.4%) and 10 females (9.6%) underwent clinical examinations in the hospital. All the people were asked to provide a written consent for the medical examinations and the study. Patients were then questioned about the consumption of the lunch cooked using polluted water, presence of recent symptoms, pharmacological treatment at home and any disease history, and following tests were carried out in the hospital: (1) blood cell counts using a AC-900 hematology analyzer (Swarovski, Sweden); (2) biochemistry tests of liver, kidney, and heart function using an AU-400 autoanalyzer (Olympus, Japan); (3) ultrasound examination of liver, gallbladder and spleen using a SSH-140A CE imaging system (Toshiba, Japan); (4) cardiac function using an ECG-6511 electrocardiograph (Nihon Konden, Japan); and (5) nerve conduction studies using an electromyogram/evoked potential systems (Keypoint, Danmark). The quantitative analysis of total arsenic (TAs) in the urine was performed by the Center for Disease Control and Prevention in Fuxin with AA-6650 atomic absorption spectrometry (Shimadzu, Japan).

**Chronic high arsenic–exposed and control subjects.** The chronic high arsenic–exposed population was formed by 72 males (mean age, 32 years old; range, 18–73 years old) from Shiligu village, near Hohhot, Inner Mongolia, China. All residents in this village were exposed to 0.24 mg/L arsenic in drinking water for 6 years. The control group with 40 males (mean age, 29 years old; range, 18–60 years old) was from the Tianjiaying village on the outskirts of Hohhot where the deep tube-well water was provided by centralized waterworks to community with iAs of 20 μg/L. Drinking water standard for iAs is 50 μg/L in China at present.

This study was approved by the Ethics Committee of China Medical University and conducted according to the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. All the villagers gave informed consent before participating. The subjects were generally healthy without any history of other chronic diseases.

**Sample collection.** Urine samples for 76 of the 104 patients were collected prior to any therapeutic intervention after they were admitted to the hospital. We failed to collect urine samples for the rest 28 patients. For the chronic high arsenic–exposed and control subjects, spot urine samples (10 ml) were collected. Each urine sample was collected in a 15-mL polypropylene tube (Sarstedt, Japan), transported on dry ice and stored at −80°C before analysis.

**Determination of urinary arsenic species.** Determination of arsenic species, including iAs, MMA, DMA, and trimethylated arsenic (TMA) in urine was performed by AA-6800 atomic absorption spectrophotometer (Shimadzu) with an arsenic speciation pretreatment system ASA-2p (Shimadzu) in our laboratory. Arsenic speciation was based on the well-established hydride generation of volatile arsines, followed by cryogenic separation in liquid nitrogen. The absorbance of arsenic in the digested urine samples was determined at 193.7 nm. The detection limit of the hydride generation and atomic absorption spectrometry method for four arsenic species is 1 ng. Briefly, urine thawed at room temperature was digested with 2N-NaOH at 100°C for 3 h followed by dilution with Milli-Q water. The assay samples were stirred once every 60 min. This digestion procedure has been shown not to alter the distribution of iAs and methylated arsenicals (Yamauchi and Yamamura, 1984).

Quality control for arsenic determinations included the analysis of Standard Reference Material of freeze-dried urine (SRM 2670). The certified average concentration values for arsenic were 480 ± 100 μg/L. The average value measured in our laboratory was 464 ± 21 μg/L. The reliability of arsenic species separation was evaluated by the analytical recoveries of added arsenic species. Spiking urine samples with 10 μg/L of iAs, MMA, DMA, and TMA resulted in recoveries of 85–98%, 84–92%, 90–105%, and 81–96%, respectively. Under our analytical conditions, differentiation of the trivalent forms from the pentavalent forms of arsenic cannot be performed. The concentrations of arsenicals in urine were corrected by individual urinary concentration of creatinine (Cr). We reported the TAs concentrations by summing up the concentrations of iAs, MMA, DMA, and TMA. Relative Occurrence of Methylated Arsenic Metabolites in Urine was assessed by the proportions of iAs, MMA, and DMA to TAs.

**Data analysis.** Statistical analysis was conducted by using the SPSS software (version 11.5; SPSS, Inc., Chicago, IL). Mann–Whitney U test was used to determine the statistical significance for the differences of urinary arsenic levels between patients with and without TMA in urine. One-way ANOVA and Student-Newman-Keuls post hoc test were performed to determine the statistical significance for the differences of urinary arsenic concentrations and proportions among subacute arsenic poisoning, chronic high arsenic–exposed and control population. All p values were two-tailed.

**RESULTS**

**Clinical Symptoms**

All patients had normal vital signs. Initial clinical symptoms of most patients (83 people, 79.8%) related to the gastrointestinal system were burning pain in esophagus and pharynx, nausea, vomiting, abdominal pain, and watery diarrhea of varying degrees. Four people (3.8%) even presented with “bloody rice water” diarrhea. Other clinical symptoms included headache, vertigo, weakness, facial edema, coughing, dyspnea, and pain in testes. The other 21 people (20.2%) had no gastrointestinal symptoms. The distribution of initial symptoms is presented in Table 1. These effects continued
from day 2 to day 7 after the onset of chelation therapy and supportive care. Within 5–15 days after admission, five people (4.8%) developed red papular eruptions, scattered on the back, buttocks, abdomen, chest and arms, and two people (1.9%) exhibited fine bran-like desquamation of the skin mainly on the trunk. Hyperpigmentation, keratosis, or Mee’s lines were not observed. Seventeen days after admission, several patients began to develop peripheral neuropathy, including limb weakness (19 people, 18.3%), prickly paresthesia (one person, 1.0%), convulsive pain in gastrocnemius muscle (three people, 2.9%), and numbness or weakness in a distal to proximal gradient in a glove-and-stocking distribution (22 people, 21.15%).

**Laboratory Tests, Treatment, and Recovery**

Table 2 shows the results of laboratory tests. Ninety-seven percent patients had elevated urinary arsenic, which reflected their high exposure to arsenic. A total of 69.2% patients showed decreased white blood cells (WBC). More than 50% patients were increased in alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and 25–40% patients were increased in lactate dehydrogenase (LDH), alkaline phosphatase (ALP), \( \gamma \)-glutamyltransferase (GGT), and fasting plasma glucose (FPG), which indicate acute liver injury in the accident. The presence of hematuria was also in a significant portion of the patients, as much as 33.6%. The decreased red blood cells (RBC) and elevated blood urea nitrogen (BUN) were only observed in a few cases, 4.8% and 1.9%, respectively. Electrocardiograph analysis revealed myocardial ischemia in one patient (1.0%). Ultrasound-examinations were unremarkable. Seventeen days later, electromyogram demonstrated the decrease of motor and sensory nerve conduction velocity in 25 people (24.0%).

All 104 patients immediately received chelation therapy with dimercaptopropanesulfonic acid (DMPS) after diagnosed with arsenic poisoning. Each patient was intravenously given 250 mg DMPS per day until his urinary arsenic excretion dropped into the normal range (0.005-0.05 mg/L) (Goyer and Clarkson, 2002). All patients were asked to refrain from seafood to avoid organic arsenic and urinary arsenic fell back to normal in 25–40 days. Colloidal bismuth subcitrate and sodium bicarbonate were administered to protect gastric mucosa and maintain an alkaline diuresis in patients with serious gastrointestinal symptoms. Vitamin B group and trace elements were also administered. Limb massage and exercise were conducted to prevent myophagism. Within 7 weeks of admission, the values of all laboratory tests in the 104 patients were normal. All patients recovered from peripheral neuropathy in 40 weeks.

**Urinary Arsenic Species and TAs Concentrations**

Arsenic species as well as TAs content of urine samples of the 76 people (72 males and 4 females) with subacute arsenic poisoning are shown in Table 3. Three arsenic species that are

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**TABLE 1**

**Distribution of Initial Clinical Symptoms in 104 Subacute Arsenic-Poisoned Patients**

<table>
<thead>
<tr>
<th>Patients with gastrointestinal symptoms*, ((n = 83))</th>
<th>Patients without gastrointestinal symptoms, ((n = 21))</th>
<th>Total, ((n = 104))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(n)</strong></td>
<td><strong>%</strong></td>
<td><strong>(n)</strong></td>
</tr>
<tr>
<td>Headache, vertigo</td>
<td>35</td>
<td>42.2</td>
</tr>
<tr>
<td>Weakness</td>
<td>33</td>
<td>39.8</td>
</tr>
<tr>
<td>Facial edema</td>
<td>20</td>
<td>24.1</td>
</tr>
<tr>
<td>Coughing, dyspnea</td>
<td>13</td>
<td>15.7</td>
</tr>
<tr>
<td>Pain in testes</td>
<td>2</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*Gastrointestinal symptoms include burning pain in esophagus and pharynx, nausea, vomiting, abdominal pain, and watery diarrhea of varying degrees.

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**TABLE 2**

**Abnormal Cases in Laboratory Tests**

<table>
<thead>
<tr>
<th>Items</th>
<th>Abnormal cases, ((n, %))</th>
<th>Abnormal range</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary arsenic ((\text{mg/L}))</td>
<td>101, 97.1%</td>
<td>0.2–12.7</td>
<td>0.005–0.05</td>
</tr>
<tr>
<td>WBC ((10^9/L))</td>
<td>72, 69.2%</td>
<td>1.4–3.3</td>
<td>4–10</td>
</tr>
<tr>
<td>RBC ((10^{12}/L))</td>
<td>5, 4.8%</td>
<td>1.5–3.0</td>
<td>4.0–5.5</td>
</tr>
<tr>
<td>ALT ((U/L))</td>
<td>84, 80.8%</td>
<td>41–1005</td>
<td>0–40</td>
</tr>
<tr>
<td>AST ((U/L))</td>
<td>58, 55.8%</td>
<td>41–537</td>
<td>0–40</td>
</tr>
<tr>
<td>LDH ((U/L))</td>
<td>38, 36.5%</td>
<td>244–592</td>
<td>114–240</td>
</tr>
<tr>
<td>ALP ((U/L))</td>
<td>30, 28.8%</td>
<td>102–143</td>
<td>34–100</td>
</tr>
<tr>
<td>GGT ((U/L))</td>
<td>26, 25.0%</td>
<td>55–235</td>
<td>5–50</td>
</tr>
<tr>
<td>BUN ((\text{mmol/L}))</td>
<td>2, 1.9%</td>
<td>10.2–11.6</td>
<td>3.2–7.1</td>
</tr>
<tr>
<td>Urinary RBC ((/\text{high-power field}))</td>
<td>35, 33.6%</td>
<td>5–18</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>FPG ((\text{mmol/L}))</td>
<td>36, 34.6%</td>
<td>6.2–15.4</td>
<td>3.5–6.1</td>
</tr>
<tr>
<td>Serum creatinine ((\mu\text{mol/L}))</td>
<td>0 —</td>
<td>88.4–176.8</td>
<td></td>
</tr>
<tr>
<td>Urinary creatinine ((\mu\text{mol/L}))</td>
<td>0 —</td>
<td>6.2–13.3</td>
<td></td>
</tr>
</tbody>
</table>

*Not detected.
to be expected in human urine (iAs, MMA, DMA) were detected in all 76 samples. TMA was detected only in eight urine samples. The average concentrations of urinary iAs, MMA, DMA, and TAs were all higher for the eight patients with TMA in urine compared with the 63 patients without TMA in urine ($p = 0.029, 0.039, 0.036, \text{and } 0.017, \text{respectively}$).

**Relative Occurrence of Arsenic Metabolites in Urine**

For analysis of relative occurrence of urinary arsenic metabolites in subacute arsenic poisoning, 8 of the 76 urine samples were excluded from the study because they contained TMA. Because there were so few female patients, their urines were also excluded. All the rest 65 urine samples belonging to male patients were analyzed. Figure 1 shows the comparison of concentrations and relative occurrence of arsenic metabolites in urine between subacute arsenic-poisoned patients, chronic high arsenic-exposed subjects and controls. Among the three groups, iAs and MMA but reduced proportion of DMA in urine compared with control subjects.

**DISCUSSION**

Arsenic, a naturally occurring metalloid in soil and water, is often used in industry and agriculture. And it is also one of the most common sources of poisoning in the world and subsequently important in the field of toxicology (Bartolome et al., 1999; Hantson et al., 2003). The toxicity of arsenic compounds varies depending on the valency, the chemical form and the physical state of the compound (Lech and Trela, 2005). Trivalent arsenic and its highly soluble forms are more toxic than pentavalent and nonsoluble forms (Quatrehomme et al., 1992). The lethal dose of arsenic trioxide in acute poisoning ranges from 1–3 mg/kg body weight for a normal adult (Heinrich-Ramm et al., 2003; Schoolmeester and White, 1980). In humans, the ingested iAs undergoes metabolic conversion that generates trivalent and pentavalent methylated arsenicals (Aposhian, 1997). The trivalent monomethylated and dimethylated arsenic species were reported to be more toxic than the pentavalent and nonsoluble forms (Dopp et al., 2004; Petrick et al., 2000; Styblo et al., 1999, 2000). Arsenic poisoning in our study was caused by arsenic trioxide in industrial waste generated from smelting of arsenic-containing mineral. The only way for...
arsenic exposure in this current study subjects (workers in the factory) was by consumption of lunch made using arsenic polluted well water. The exact amount of ingested arsenic was difficult to know, but can be estimated. About 120–130 L (12–13 buckets) well water was used to make lunch for 250 workers every day. Each worker ingested about 0.5 L polluted well water containing 24 mg arsenic from rice, vegetables, meat and soup in lunch every day. The workers presented with symptoms of subacute arsenic poisoning after 5–8 continuous days of taking such meals, with estimated cumulative arsenic exposure of 121–194 mg. The average level of urinary TAs for patients when they were admitted into hospital was 87.5 times that of the control group (0.0095 mg/L). Drinking water of 12 families around the factory was supplied by the polluted well. So the family members might have ingested more arsenic, and the symptoms of arsenic poisoning for them showed up earlier and were more serious. However, this study only included factory workers.

It is generally recognized that one mechanism of arsenite toxicity is its ability to bind with sulfhydryl (SH) groups, resulting in the dysfunction of enzymes in cellular energy pathways and DNA replication and repair (Ratnaike, 2003). This causes capillary damage, and leads to generalized vasodilatation and transudation of plasma, and with high levels, shock (Bartolome et al., 1999; Lech and Trela, 2005). Acute arsenic toxicity is characterized by severe gastrointestinal symptoms, which occur from 30 min to several hours after ingestion of a lethal dose. Eventually, severe gastrointestinal hemorrhaging occurs, leading to vascular collapse, shock, and death. If this initial toxic phase is survived, jaundice, renal failure, and peripheral neuropathy may develop (Merrill et al., 2001). Chronic exposure to arsenic from drinking water may lead to neurotoxicity in both peripheral and central nervous systems, skin lesions such as keratosis, pigmentation and Bowen’s diseases, liver injury and cancers of various organs (Goyer and Clarkson, 2002).

There are few reports on subacute arsenic poisoning, in which extremely high dose of arsenic was ingested in a short term, so the systematic clinical data in the present work is very valuable. In our study, most patients (83 people, 79.8%) initially presented with gastrointestinal symptoms after 5–8 days of continuous arsenic ingestion. Though diarrhea was predominant, it was not as serious as in acute fatal poisoning. Headache, vertigo, weakness, and facial edema were the main complaints besides the gastrointestinal symptoms. Only 24% of patients with gastrointestinal symptoms exhibited facial edema, whereas 43% of patients without gastrointestinal symptoms had facial edema. Though the difference was not statistically significant, it suggested different degree of dehydration by diarrhea in these two groups of patients. Laboratory tests indicated that leucopenia and mild hepatic injury occurred in most patients, and RBC reduction, FPG elevation and renal damage in a few cases. 33.6% patients were presented with hematuria, which indicate acute urinary injury of arsenic. The only case with electrocardiographic abnormality had a history of heart disease. The levels of urinary arsenic and laboratory tests were normal for 3 of the 104 people, but they none-the-less complained of symptoms related to arsenic poisoning. A psychosomatic origin of the symptoms in this subgroup caused by publicity of the poisoning could not be excluded. After the initial phase, 6.7% of the patients developed cutaneous signs of arsenic poisoning and 22.2% of the patients developed peripheral neuropathy. Though the symptoms of the subacute arsenic poisoning were to some extent similar to acute arsenic poisoning reported in the literature (Isbister et al., 2004; Lech and Trela, 2005; Nakawatase and Nakatsuka, 1993; Ratnaike, 2003; Seavolt et al., 2002), they were not life threatening.

Treatment of acute or subacute arsenic poisoning includes individual decontamination, chelation therapy and supportive care. Various methods of arsenic decontamination such as absorption to activated charcoal, gastric lavage with repetitive endoscopy, and bowel irrigation have been used in cases of arsenic poisoning (Isbister et al., 2004; Lee et al., 1995; Michaux et al., 2000). However, the effects of these methods are not well defined. It is unclear if invasive decontamination procedures affect the clinical outcome in patients poisoned with arsenic beyond chelation therapy. In the present case decontamination treatment was not used. Treatment with SH-containing chelators such as DMPS, meso-2,3-dimercaptosuccinic acid or British anti-Lewisite, 2,3-dimercaptopropanol (BAL) are the usual choice in chelation therapy for iAs intoxication. These dimercapto chelators compete with SH groups in tissues or enzymes for binding arsenic, which results in a more effective elimination of the metalloid (Heinrich-Ramm et al., 2003). BAL has been shown to cause adverse effects and increase deposition of arsenic in the central nervous system (Muckter et al., 1997). DMPS is a good choice for chelation of arsenic because: (1) it has a high therapeutic index for experimental animals treated with lethal dose of arsenic trioxide (Aposhian et al., 1984); (2) DMPS is water-soluble and may be administered orally or intravenously (Hurlbut et al., 1994; Muckter et al., 1997); (3) DMPS is rapidly eliminated via the kidneys with a half-life of 20 h (Heinrich-Ramm et al., 2003; Hurlbut et al., 1994). In our study, chelation therapy with DMPS was commenced as recommended, initiated as soon as possible and continued until the urinary excretion levels of arsenic were reduced to the normal range (Graeme and Pollack, 1998).

About 80–90% of ingested iAs has been shown to be absorbed from gastrointestinal tract of humans and experimental animals (Goyer and Clarkson, 2002). The primary metabolic pathway of iAs in humans is methylation to MMA and DMA in multiple steps by methyltransferases. This enzymatic process occurs mainly in the liver but also in kidney, lungs and testes (Ford, 2002). Urinary arsenic level is the best indicator of exposure because it is the main route of excretion for most arsenicals (Goyer and Clarkson, 2002). Analyses of relative occurrence of urinary arsenic metabolites can provide insight...
into the methylation capacity of exposed individuals. Most human subjects exposed to arsenic from environment or at work have shown average values of 10–30% inorganic, 10–20% MMA, and 60–70% DMA in urine (Vahter, 2000). However, there is a considerable interindividual variation in arsenic methylation due to factors such as possible genetic polymorphisms, age, sex, and nutrition (Sun et al., 2007). Variability of arsenic methylation capacity has been suggested to be associated with arsenic-induced diseases (Tseng, 2007). So it is of great importance to assess arsenic methylation in an exposed population. Prior assessment of arsenic methylation patterns was not available in subacute arsenic poisoning. Because DMPS can inhibit the secondary methylation step of arsenic (Heinrich-Ramm et al., 2003), to assess effects of subacute exposure on arsenic methylation, only urine samples taken before chelator treatment were analyzed in this study. Of the three populations we reported in our study, the ingested dose rate of arsenic was highest for subacute arsenic-poisoned subjects, relatively lower for chronic exposed subjects and lowest for controls. The relative occurrence of urinary iAs and MMA (iAs% and MMA%) responded to increase in dose rate of ingested arsenic in a similar fashion. In contrast, with increased dose rate of arsenic, DMA% in urine progressively decreased. These results suggested that the arsenic methylation capacity was impaired by ingested arsenic in a dose-rate–dependent manner. This dose-dependent decrease of arsenic biomethylation was previously observed by Csanaky et al. (2003) in arsenite-injected rats. The striking decrease of arsenic methylation capacity may be caused by the inhibition of arsenic methyltransferase or the depletion of glutathione, which are required for arsenic methylation. Though a considerable interindividual variation of arsenic methylation capacity was observed in the subacute poisoned subjects, correlations between arsenic methylation capacity and symptoms were not observed. On average, patients with TMA detected in urine had significantly higher concentrations of arsenic metabolites in urine compared with those without TMA in urine. However, one patient with extremely high concentration of urinary arsenic, as much as 7258.39 mg/g Cr, had no TMA in urine. In addition, considering the small sample size (eight patients with TMA in urine), the connection of urinary TMA with the high arsenic methylation at high dose.

In summary, we reported the clinical features, therapeutics and the striking alteration of the relative occurrence of urinary arsenic metabolites in a subacute arsenic poisoning accident. Though uncommon, this may not be an isolated one, and the environmental protection and medical communities should be alerted to the signs and symptoms of subacute arsenic poisoning. Finally, smelting factories should be built far from the public drinking water sources and residential areas.

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