Acute arsine poisoning confirmed by speciation analysis of arsenic compounds in the plasma and urine by HPLC-ICP-MS

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**Abstract**

Objectives: Arsine, a potent hemolytic agent, is widely used in the semiconductor industry. We report a case of arsine poisoning confirmed by arsenic speciation analysis in serum and urine that occurred in a recycling factory. Case: A male worker in his twenties noticed hematuria 3 h after finishing work and was admitted into our hospital 34 h later. Speciation analysis of arsenics in serum and urine samples was performed using HPLC-ICP-MS. On admission, anemia, hematuria, and renal and liver dysfunction were observed. His clinical condition had improved remarkably after 5-days of transfusion and 4 units of RBC transfusion. The total arsenic content in the serum was 244.8 µg/l at admission and 97.1 µg/l at discharge. In the speciation analysis, four kinds of As compounds derived from arsine metabolism were detected in serum and urine. The concentrations of arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in serum at admission were 45.8, 5.2, 17.9 and 9.3 µg/l, respectively. The concentrations of AsIII, AsV and MMA decreased with biological half-lives (BHL) of 30.1, 43.0, and 96.3 h, respectively, while that of DMA was increased towards discharge. The urinary AsIII, AsV, MMA, and DMA concentrations at discharge were 384.5, 20.8, 547.4 and 1816.3 µg/g creatinine, respectively. These concentrations in urine subsequently decreased and their BHL was 15 d. Conclusion: The results of the present study suggest that arsine is quickly metabolized to AsIII and then metabolized via MMA to DMA in humans.

Key words: Arsenic compounds, arsine, gallium, plasma, speciation analysis, urine

**Introduction**

Arsine is a colorless gas and one of the most potent hemolytic agents used in industry. Arsine is extensively used for epitaxial growth of gallium arsenide (GaAs) and as a dopant for silicon-based electronic devices in the semiconductor industry\(^1\).

Scrap recycling accounts for 40% of the total production of high-purity gallium in Japan\(^2\). Arsine generation may occur during the recycling process of various grades of GaAs scraps. Here, we report a case of arsine poisoning that occurred in a recycling factory.

**Case**

A man in his twenties visited our hospital emergency room because of hematuria and vomiting; he was immediately admitted.

On admission, his temperature was 37.5°C, bulbar conjunctiva was icteric, palpebral conjunctiva was not anemic, and pharynx was reddish in color. His breath sounds were normal, and there were no crackles or wheezes. Other prominent physical findings were normal. Anemia, renal and liver dysfunction, and hematuria were confirmed on the basis of laboratory analysis of blood and urine samples (Table 1). Blood urea nitrogen (BUN) level was 39.8 mg/dl. The levels of
inflammatory markers, such as white blood cells (14300/µl), creatine kinase (406 U/l), and C-reactive protein (7.0 mg/dl) were high. The patient provided informed consent for publishing a case report.

Since the patient could not provide any information about arsine exposure, initially, we suspected that his hemolytic anemia had been caused by a mycoplasma or viral infection and therefore started supportive treatment such as intravenous (IV) therapy. His clinical course and laboratory findings are described in Table 1. His hemoglobin value decreased to 5.9 g/dl on day 3; therefore, blood transfusion was performed (2 units of packed red blood cells daily for 2 days). His general condition, anemia, and other laboratory data improved, and he was discharged on day 7. When he visited our clinic after discharge (day 18), his physical condition was good, and his laboratory data were within the normal ranges, except for slightly elevated serum creatinine (CRE) level; therefore, we allowed him to resume work. When he visited our clinic for examinations on days 68 and 110, his anemia, hematuria, renal dysfunction, and liver injury had completely disappeared, and no exacerbation was observed.

**Work environment and working condition**

The patient started working in a factory, which extracts of Ga from GaAs scraps, 3 months before the accident. At the factory, unused residue of GaAs-epitaxial substrate containing Ga-As solution is mixed with water and crushed in a ball-mill for 12 h. Degassing is necessary to release the excess pressure that develops during crushing.

The patient worked on a crusher for 1–2 h a day 2–3 days per week. He wore protective gloves and a gas respirator to prevent inhalation of acid gases. He worked with another worker but the worker did not complain of any symptoms.

He first noticed hematuria 3 h after completing a 2-h work shift and was admitted to our hospital 34 h later. After discharge, he was transferred to a workshop with no arsine exposure.

In order to gather more information in regard to his working situation, industrial hygienists at the factory reproduced his accidental situation and exposure to arsine gas. It was considered that arsine gas was produced by As reduction and Ga oxidization from the mixture of Ga-As solution and water, because the ionization tendency of As is lower than that of Ga. We confirmed that he was accidentally exposed to arsine gas during the degassing process. Therefore, the gas respirators of the workers involved in the degassing process were replaced with air-line respirators.

**Determination of arsenic compounds in serum and urine**

Total arsenic (T-As) and Ga concentrations in serum were determined using an Agilent 7500cx ICP-MS fitted with an octopole-based collision/reaction cell (Agilent Technologies, Santa Clara, USA). The limits of detection (LOD) for As and Ga were 0.005 and 0.007 µg/l, respectively.

We observed a significant decrease in the T-As concentration with time and estimated its
biological half-life (BHL) to be 59.2 h. The concentrations of Ga in serum measured at 34 and 113 h were <0.14 and 0.59 µg/l, respectively.

Arsenic speciation analysis was performed according to our previously reported method after deproteinization with Microcon YM-10 (Millipore, MA, USA). The recovery rates for As species from the filtration ranged from 99 to 106%.

Arsenic speciation analysis of the serum and urine samples revealed the presence of 4 kinds of As compounds derived from arsenic metabolism and arsenobetaine (AsBe). The variations in serum concentrations of As species during hospitalization are shown in Table 1. We found that arsenite (AsIII) had the highest concentration at 34 h. It had a short BHL of 30.1 h. All 3 arsenate (AsV) concentrations were much lower than those of AsIII, and their BHL was slightly longer at 43.0 h. The concentration of methylarsonic acid (MMA) showed a slight increase, but it decreased later; its BHL was estimated to be 96.3 h. The concentration of dimethylarsinic acid (DMA) increased with time during the 6-day hospitalization; hence, its BHL could not be calculated. The patient’s AsBe concentration was almost constant throughout his hospitalization.

The variations in urinary arsenic concentrations are described in Table 1. The sum of AsIII, AsV, MMA, and DMA concentrations sampled 6 days after the accident was 29-fold higher than that of the 95 percentile reference value of 96.7 µg/g CRE obtained in our previous study. The urinary concentrations of all the As compounds, except AsBe, subsequently decreased; and their BHL was 15 d. The urinary Ga concentrations at 6, 68, and 110 days after the accident were <0.24, 3.79, and 0.57 µg/g CRE, respectively.

**Discussion**

Speciation analysis revealed significant amounts of arsenic compounds in the patient’s serum and urine samples; therefore, we diagnosed the patient with acute arsine poisoning. An experiment conducted at the factory also confirmed the generation of arsine.

Symptoms of arsine poisoning appear 1–24 h after exposure, depending on the concentration and time of exposure. Our patient showed symptoms of poisoning 3 h after exposed to arsine for a duration of 2 h. His serum T-As concentration was 244.8 µg/l. He also developed anemia, renal and liver dysfunction, and hematuria. He did not develop acute oliguric renal failure, and therefore, exchange transfusion was not required. He was adequately treated by early IV fluid therapy and RBC transfusion for severe anemia.

This is the first report of speciation analysis of arsenic in serum from a patient with acute arsine poisoning. In the speciation analysis of the patient’s serum, we detected 4 kinds of As compounds derived from arsenic metabolism and AsBe. Although we used 2 different columns, we did not detect other forms of As compounds in the serum. Yoshino et al. detected arsenite (AsIII), arsenate, MMA and DMA, in the plasma of a patient who received arsenic trioxide treatment, and reported that T-As in
blood cells were 4-10 times higher than those in plasma. The proportion of the sum of the concentrations of the four As species, expressed as extracted As (E-As), to T-As were constant in their patient, while, it increased with time from 33.3% at 34 h to 53.5% at 112 h in our patient. When a human blood sample was exposed to arsine vapor, partial hemolysis was observed, and As–adducts were detected in the plasma. Since our patient had hemolytic anemia, T-As in his serum sampled at 34 hours after the accidental exposure may have contained As-adducts bound to hemoglobin. Thus, the proportion of E-As to T-As in our arsine poisoning patient was remarkably different from that in the patient exposed to AsIII. This may be a characteristic of the metabolic profile of arsine poisoning.

Our results indicated that inhaled arsine is rapidly converted to AsIII and then metabolized via MMA to DMA. Apostoli et al. reported that the BHL of T-As in the blood of a patient with acute arsine poisoning followed a triphasic model, with periods of 28 h, 59 h, and 9 d. Our estimated BHL of T-As in serum (59.2 h) is in good agreement with their second-phase BHL of T-As in blood. Among the As compounds, DMA alone had serum concentrations that showed a progressive increase up to 112 h. The possible reason for this finding is as follows: arsine itself, and/or excessive AsIII, and its metabolite may suppress the second methylation step from MMA to DMA that is mainly mediated by hepatic enzymes. The presence of liver dysfunction in the patient also supports this possibility. In contrast, the BHLs of urinary As species were calculated to be 15–20 d. These BHLs are similar to the third-phase BHL of serum T-As reported by Apostoli et al.

Ga concentrations in serum and urine were within the reference range. Since prominent acute respiratory symptoms were absent, we think that the patient did not inhale a significant amount of GaAs.

Ingestion of seafood including seaweeds increases urinary T-As and DMA concentrations. The patient’s serum and urine AsBe concentrations were low, and hence, seafood ingestion was not considered to have had any effect on his T-As and DMA concentrations. On the basis of the hypothesis the patient’s urinary As level decreased with the serum T-As BHL of 59.2 h before and during hospitalization and then with the urinary T-As BHL of 15 d after hospitalization, we estimated that the patient had excreted approximately 78 mg of As. A previous study had estimated the pharmacokinetic parameters by determining the blood level of inorganic As (iAs) in patients administered arsenic trioxide; on the basis of this study, we found that our patient (body weight, 58.4 kg) had absorbed approximately 63 mg of As. Both the estimates yielded almost identical values. This finding indicates that the patient could have been exposed to about 28–39 mg/m³ (9–12 ppm) of arsine. The level of exposure is about 100 times higher than the Occupational Exposure Limit-Ceiling of 0.1 ppm recommended by The Japan Society for Occupational Health and is high enough to cause arsine poisoning, because a previous study have reported the appearance of symptoms of poisoning after a few hours of exposure at 1–5 ppm.

In conclusion, accidental arsine poisoning occurred in a GaAs recycling factory. Although
arsine toxicity is very different from iAs toxicity, the characteristics of the metabolites resulting from arsine exposure were considered to be similar to those of the metabolites resulting from iAs exposure. The effects of iAs toxicity should be considered in chronic arsine exposure. Therefore, in order to assess the adverse effects of arsine exposure, toxicity due to iAs exposure appearing in other than the hematopoietic system should be fully considered and their preventive measures should be adopted not only for acute toxicity but also for chronic toxicity.

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References


<table>
<thead>
<tr>
<th>Laboratory values</th>
<th>Reference Range</th>
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<td>RBC (x10^6/mcL)</td>
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<tr>
<td>Hb (g/dL)</td>
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<td>AST (U/L)</td>
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<td>Total bilirubin (mg/dL)</td>
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<td>Direct bilirubin (mg/dL)</td>
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<tr>
<td>Protein (g/dL)</td>
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<tr>
<td>Albumin (g/dL)</td>
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</tbody>
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Table 1: Timed course of laboratory values and vs concentrations in serum and urine and clinical course of the patient with acute ascending cholangitis.