A biological indicator of inorganic arsenic exposure using the sum of urinary inorganic arsenic and monomethylarsonic acid concentrations

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Abstract: Objectives: The sum of urinary inorganic arsenic (iAs), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) concentrations is used for the biological monitoring of occupational iAs exposure. Although DMA is a major metabolite of iAs, it is an inadequate index because high DMA levels are present in urine after seafood consumption. We estimated the urinary iAs+MMA concentration corresponding to iAs exposure.

Methods: We used data from two arsenic speciation analyses of urine samples from 330 Bangladeshi with oral iAs exposure and 172 Japanese workers without occupational iAs exposure using high-performance liquid chromatography with inductively coupled plasma mass spectrometry. Results: iAs, MMA, and DMA, but not arsenobetaine (AsBe), were detected in the urine of the Bangladeshi subjects. The correlation between iAs+MMA+DMA and iAs+MMA was obtained as log (iAs+MMA) = 1.038 log (iAs+MMA+DMA)−0.658. Using the regression formula, the iAs+MMA value was calculated as 2.15 and 7.5 μg As/l, corresponding to 3 and 10 μg As/m³ of exposures, respectively. In the urine of the Japanese workers, arsenic was mostly excreted as AsBe. We used the 95th percentile of iAs+MMA (12.6 μg As/l) as the background value. The sum of the calculated and background values can be used as a biological indicator of iAs exposure. Conclusion: We propose 14.8 and 20.1 μg As/l of urinary iAs+MMA as the biological indicators of 3 and 10 μg As/m³ iAs exposure, respectively. (J Occup Health 2016; 58: 196-200) doi: 10.1539/joh.15-0241-OA

Key words: Arsenic, Biological monitoring, Occupational exposure, Oral exposure, Speciation

Introduction

Arsenic comes from mineral weathering and volcanic activity and is carried to the sea by groundwater and rivers. Inorganic arsenic (iAs) is found in drinking water, and an organic arsenic compound in addition to iAs is found in food. iAs species are metabolized to monomethylarsonic acid (MMA) and subsequently to dimethylarsinic acid (DMA) in humans¹. The total concentration of iAs and its metabolites, MMA and DMA, excreted in urine has been used for the biological monitoring of occupational iAs exposure by the American Conference of Governmental Industrial Hygienists (ACGIH) and Deutsche Forschungsgemeinschaft (DFG)²³). In addition to DMA, methylated arsenics such as arsenobetaine (AsBe), arsenocholine (AsCho), and arsenosugars (AsSugs) are abundant in seafood products⁴⁵. Although AsBe is only minimally metabolized in mammals⁶, AsCho and AsSug are extensively metabolized to AsBe and DMA⁷⁸, respectively. Therefore, large amounts of AsBe and DMA and small amounts of MMA and iAs have been observed in the urine of seafood consumers without occupational iAs exposure⁹¹⁰. In our previous reports, for instance, the me-
had a mean age of 38.8±11.0 years (range, 20-77 years), whereas the 172 Japanese participants were healthy male workers with a mean age of 46.5±13.6 years (range, 18-74 years) who had no occupational arsenic exposure for at least 6 months. Urine samples were collected and stored as described in our previous reports. These studies were approved by the relevant ethics committees.

**Arsenic speciation analysis**

A urinary arsenic speciation analysis was performed using high-performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry. The details, including the chemicals used, sample preparation, analytical conditions, and analytical procedure validation, are described in our previous reports.

**Extrapolation of urinary arsenic concentration resulting from oral exposure to inhalation exposure**

The Japanese government ministry defines the sum of iAs and MMA levels in the urine as the iAs exposure indicator, and its measurement is the second item of the special health examination of hazardous chemicals. The Japanese Administrative Level is set at 3 μg As/m³ of iAs exposure. An extrapolation is performed under the following assumptions used by ACGIH: 1) pulmonary ventilation is 10 m³/8 h; 2) absorption is 60%; 3) 60% of the absorbed dose is recovered in urine; 4) urinary arsenic concentration remains consistent during the day; and 5) daily urine output is 1.2 l.

Calculations were performed using the SPSS statistical package (PASW SPSS Statistic version 18; IBM Japan, Tokyo, Japan)

**Results**

In a Bangladeshi study, arsenite (AsIII), arsenate (AsV), MMA, and DMA were detected in urine samples, whereas AsBe, which is excreted as a result of seafood ingestion, was not detected. The unidentified arsenic compounds are together termed “others.” The median concentrations (95th percentile) of AsIII, AsV, MMA, DMA, and others were 16.8 (82.2), 1.8 (9.2), 13.7 (75.4), 88.6 (381.2), and <0.5 (1.9) μg As/l, respectively. DMA was the most abundant compound, followed by AsIII, MMA, AsV, and others.

No significant differences were found in the ratios of iAs, MMA, and DMA between sexes or among subjects of different ages. Therefore, we combined the data for men and women. Fig. 1 presents the scatter plots of the most abundant compound, followed by AsIII, MMA, AsV, and others.

**Subjects and Methods**

**Subjects**

We used the following two different populations: 1) the residents of 17 villages in the Pabna District of Bangladesh who were chronically exposed to inorganic arsenic through drinking water; and 2) Japanese male workers without occupational iAs exposure. The profiles of these subjects were described in our previous reports. The 330 Bangladeshi participants (165 men and 165 women)
The median concentrations (95th percentile) of AsIII, AsV, MMA, DMA, AsBe, and others were 1.5 (5.4), <0.5 (1.7), 2.3 (6.2), 41.1 (109.2), 74.5 (120.5), and 4.1 (23.7) μg As/l, respectively. The median and 95th percentile of the urinary iAs+MMA concentrations were 4.4 and 12.6 μg As/l, respectively. The median and 95th percentile of the urinary iAs+MMA+DMA concentrations were 7.3 μg As/day and 11.5 μg As/l, respectively. These values are within the range of the median and 95th percentile levels (4.4 and 12.6 μg As/l, respectively) reported in our previous results.

We estimated the sum of the urinary iAs and MMA concentrations at 3 μg As/m3 of exposure using the assumptions described in the Subjects and Methods section. A worker absorbs 18 μg of arsenic per day, of which 10.8 μg is excreted in the urine. A urinary concentration of 9 μg As/l is substituted for x in the equation log y = 1.038 log x − 0.658. The value of 2.15 μg As/l is obtained as y (iAs+MMA). When we consider the sum of the 95th percentile of the Japanese workers’ iAs and MMA concentration of 12.6 μg As/l to be the background level, 14.8 μg As/l is assumed to be the biological exposure index corresponding to the Japanese Administrative Level of 3 μg As/m3. Similarly, 20.1 μg As/l is assumed to be 10 μg As/m3 of iAs exposure.

Discussion

For the biological monitoring of exposure to iAs, the seafood intake should not interfere with the urinary concentrations of iAs metabolites. ACGIH and DFG use the total concentration of urinary iAs+MMA+DMA, excluding AsBe, for the biological monitoring of occupational exposure to iAs, because AsBe is the main arsenic compound in fish and shellfish. ACGIH reported that the urinary arsenic concentrations in the general population were approximately 10 μg As/l in European countries and the United States and approximately 50 μg As/l in Japan. DFG defines the Biologische Arbeitsstoff-Referenzwerte (BARs, biological reference values for workplace substances) as the background levels present at a particular time in a reference population of persons of working age who are not occupationally exposed to the substances; the BARs are based on the 95th percentile without taking the effects on health into consideration. DFG set BARs for As3+, As5+, MMA, and DMA as 0.5, 0.5, 2, and 10 μg As/l, respectively. These BARs are consistent with the 95th percentile of urinary levels obtained from 82 German adults with no occupational exposure.

By contrast, the urinary levels of arsenic of the Japanese subjects who were not occupationally exposed to iAs but were exposed to organic arsenic from food were reported to be much higher than those reported in the German data. Mohri et al. reported that the mean daily intake of dietary arsenic was 182 μg As (range, 27-376) for 4 Japanese volunteers. The mean amount of total arsenic eliminated daily in urine was 148 μg As (range, 50-416) and composed of 1.4% iAs, 3.5% MMA, and 33.6% DMA. Yamauchi et al. reported that the mean urinary total arsenic level in 56 healthy Japanese volunteers was 129±92.0 μg As/l, composed of 6.7% iAs, 2.2% MMA, and 26.7% DMA. Urinary arsenic levels in Japanese people are almost equal to or higher than 50 μg As/l of iAs+MMA+DMA. The mean concentration of iAs+MMA in urine reported by Mohri et al. and Yamauchi et al. were calculated to be 7.3 μg As/day and 11.5 μg As/l, respectively. These values are within the range of the median and 95th percentile levels (4.4 and 12.6 μg As/l, respectively) reported in our previous results.

AsSugs are the major arsenic compounds in seaweed. In particular, many studies have reported detecting DMA in urine after consumption of seaweed. AsSugs, the majority of which become DMA, are broken down in the body and then excreted. Li et al. reported that iAs were detected at levels below 2% of the total arsenic in fish or shellfish samples of Chinese seafood. However, they were not detected in algae samples. Buchet et al. found no statistically significant difference in the urinary MMA concentration between subjects who regularly consumed seafood and those who did not. Apostoli et al. observed that iAs and MMA had shorter biological half-lives than DMA. Thus, it can be concluded that seafood consumption has only a small effect on the urinary concentrations of iAs and MMA.
A simulation study presumed that 25 μg As/g of creatinine as the sum of arsenic metabolites corresponds to 10 μg As/m³ of inhalation exposure\(^3\). In this simulation, the background level was assumed to be zero.

DFG recommends 50 μg As/l as the exposure equivalent for carcinogenic substances at 0.01 mg As/m³ in the air\(^3\). Because the background level (As\(^3+\)+As\(^5+\)+MMA+DMA) established by DFG is 13 μg As/sid, the urinary excretion from inhalation exposure at 10 μg As/m³ was considered to be 37 μg As/l.

The Bangladeshi subjects in this study had no interference from seafood ingestion; therefore, they were considered to have a zero background level, and the regression formula was based on a zero background level.

Under the regression formula log \(y = 1.038 \log x - 0.658\), when we use \(x = 37 \mu g \) As/l of urinary iAs+MMA+DMA corresponding to iAs exposure at 10 μg As/m³, urinary iAs+MMA level is assigned as \(y = 9.3 \mu g \) As/l. Using the assumptions by ACGIH as mentioned in the Methods section, the urinary iAs+MMA level was estimated to be 7.5 μg As/l at 10 μg As/m³ of exposure. These concentrations were considered to be nearly comparable.

We estimated the background level of the subjects exposed to arsenic from seafood but not occupationally exposed to iAs to be 12.6 μg As/l (the 95th percentile of urinary iAs+MMA+DMA). We propose 14.8 and 20.1 μg As/l of urinary iAs+MMA for the biological monitoring of 3 and 10 μg As/m³ of iAs exposure, respectively.

This study has two limitations. First, the Bangladeshi subjects were exposed to iAs not by inhalation but through drinking water. Farmer and Johnson\(^8\) reported that the ranges in the average urinary arsenic speciation pattern in workers who occupationally inhaled inorganic arsenic were 11%-14% AsIII, 1%-6% AsV, 14%-18% MMA, and 63%-70% DMA. When we compared the urinary analysis results from various populations exposed to iAs-contaminated water using HPLC separation, the proportions of urinary iAs, MMA, and DMA were 11.4%-34.0%, 7.5%-26.9%, and 47.7%-78.8%, respectively\(^3\). Although a report that used a physiologically based pharmacodynamic model suggested that the ratio of iAs was slightly higher in those exposed to arsenic through the respiratory tract than those exposed orally\(^3\), the proportions obtained by workers who inhaled arsenic were similar to those in Asian people with oral iAs exposure. Moreover, the iAs absorption and excretion rates from repeated oral exposure was estimated to be approximately 60%-80%, and ACGIH extrapolated these rates to estimate urinary concentration resulting from inhalation exposure\(^2\). Because the normal range for 24-hour urine volume was reported to be 0.8-2.0 l\(^3\) or 1.0-1.5 l/day\(^1\), the excretion volume of urine set at 1.2 l/day under this assumption is almost the median of that of healthy adults. Thus, we can use the data of the Bangladeshi residents who were orally exposed to arsenic for the Japanese workers who were exposed to arsenic through the respiratory tract.

Second, the biological half-lives of iAs and MMA are shorter than that of DMA\(^3,10\). The sampling times of the sum of iAs, MMA, and DMA were proposed to be at the end of the workweek by ACGIH and after several shifts by DFG. Therefore, the urinary sampling time of the sum of iAs and MMA should be applied to the end of the workshift.

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