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Ingestion of Hijiki seaweed and risk of arsenic poisoning†

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The major route of human exposure to arsenic is via ingestion. Seafoods contain large amounts of various arsenic compounds. Recently, people have been advised not to eat Hijiki seaweed (*Hijikia fusiforme*) in the UK because of its high content of inorganic arsenic (iAs). To clarify the risks of Hijiki ingestion, a 42-year-old male volunteer ingested 825 μg of iAs compounds contained in eight servings of commercial Hijiki food, after refraining from eating seafood for 3 months. In order to determine the distribution of arsenic species in his urine, arsenic compounds were analyzed using HPLC-ICP-MS. The maximum concentrations of arsenate (AsV), arsenite (AsIII), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) were found at 4, 6.5, 13 and 17.5 h after ingestion, respectively. Arsenobetaine concentration was very low, and almost constant throughout the observation period. A total of 28% of ingested arsenic was excreted in urine. The total amounts of AsV, AsIII, MMA and DMA excreted in urine over 50 h were 11.2, 31.8, 40.9 and 104.0 μg, respectively. After eating one serving of Hijiki, arsenic intake and urinary excretion were at levels similar to those in individuals affected by arsenic poisoning. Long-term ingestion of Hijiki might thus have the potential to cause arsenic poisoning. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: inorganic arsenic; Hijiki; seaweed; urine

INTRODUCTION

Arsenic is widely distributed in water, air and soil.[1] Non-occupational human exposure to arsenic in the environment is primarily through ingestion in food and water.[1] Several epidemiological studies have indicated that long-term exposure to arsenic in drinking water can increase risks of cancer in the skin, lung, bladder and kidney.[2–4] However, for the general population without exposure to arsenic through occupation or an arsenic-polluted environment, food is a much more significant source of arsenic than water.[1,5–7]

The toxicity and carcinogenicity of arsenic depend on its species.[8–11] Arsenic in drinking water is a form of inorganic arsenic (iAs),[3,12] whereas seafood contains high levels of organoarsenic compounds such as arsenobetaine (AsBe), arsenocholine and arsenosugars.[13–18] Inorganic arsenic is methylated to monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and trimethylarsine oxide (TMAO) in mammals.[19–21] While AsBe is not generally biotransformed or demethylated,[22,23] arsenocholine is not demethylated but is metabolized extensively to AsBe,[24] but arsenosugars are extensively metabolized to DMA and slightly to oxo-dimethylarsenoethanol (oxo-DMAE), TMAO, and arsenothiol compounds.[1,25–28] AsBe and arsenocholine are reported not to be toxic.[1] Arsenosugars are significantly less toxic than iAs

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and not mutagenic,[29] and arsenosugar metabolites other than DMA exhibit no cytotoxicity.[28]

Recently, seaweed has become popular as a 'health food' because it is rich in dietary fiber and low in calories.[30–32] However, in July 2004, the Food Standards Agency of the UK issued an advisory against eating Hijiki (*Hijikia fusiforme*) because of its high content of iAs, although there have been no reports that ingestion of Hijiki has adverse health effects.[33] Since there are pronounced species differences in the metabolism of arsenic, it is difficult to evaluate the human metabolism of arsenic based on much of the available experimental animal data.[4] In order to clarify the metabolism of Hijiki and the risks of its ingestion, we analyzed arsenic compounds in urine after ingestion of Hijiki by a volunteer.

METHODS AND MATERIALS

Hijiki ingestion

Processed Hijiki food ('Hijiki and beans', Fujikko, Co. Ltd, Kobe, Japan) was used. One pack of this food included 165 g, and Hijiki was boiled in water with soy beans and red kidney beans. The food consisted of 55% Hijiki, 42% beans and 3% liquid, w/w. A volunteer, a 42-year-old man weighing 60 kg, consumed two 165 g packs of the whole liquid mixture of the food within 5 min. The volunteer gave informed consent and was aware of the experimental details and possible effects of ingestion of Hijiki, and the procedures followed accorded with the current revision of the World Medical Association Declaration of Helsinki.

For 3 months prior to this experiment, the volunteer refrained from eating seafood and adhered to a vegetarian diet. Urine collection was begun just after ingestion, and was continued over the next 50 h. The sampling times and volumes of urine excreted, as well as eating and drinking during the observation period, were recorded.

Chemicals

Sodium arsenite (AsIII), sodium arsenate (AsV), MMA, AsBe and NH_4NO_3 were purchased from Wako Pure Chemical (Osaka, Japan). DMA, TMAO and tetramethylarsonium iodide (TetMA) were obtained from Tri Chemical Laboratory (Yamanashi, Japan). HNO_3 of TAMA PURE-AA-10 (Tama Chemicals Co. Ltd, Tokyo, Japan) was used for the mobile phase of HPLC. 2,6-Pyridinedicarboxylic acid (PDCA) was purchased from Tokyo Kasei Industry (Tokyo, Japan). Tap water was purified through Milli-Q-ICP-MS (Millipore Japan, Tokyo, Japan) and used as super-pure water.

The certified reference material, NIES CRM no. 18 (human urine), from the National Institute for Environmental Studies, Japan, was used to validate the analytical procedure.

High-performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry (ICP-MS)

A model HP4500 ICP-MS (Agilent, USA) was used for arsenic detection. The operating conditions for ICP-MS were established in accordance with those reported by Inoue *et al*.[34] A model HP 1100 HPLC series (Agilent, USA) was used to separate arsenic species. For separation of arsenic compounds, two separation modes, cation and anion exchange, were used. The cation mode experiment, using a Shodex RSpak NN-614 (150 × 4.6 mm i.d.) packed with cation-exchange resin (Showadenko, Tokyo, Japan), was performed under the following conditions: mobile phase 5 mM HNO_3:6 mM NH_4NO_3:1.5 mM PDCA, flow rate 1.0 ml/min, ambient temperature and injection volume 50 µl. The anion mode experiment, using an Gelpack GL-IC-A15 column (150 × 4.6 mm i.d.) packed with anion exchange resin (Hitachi Resin, Tokyo, Japan), was performed under the following conditions: mobile phase 3 mM NaH_2PO_4 at pH 6 with NaOH, flow rate 0.8 ml/min, and injection volume 50 µl. The outlet from the separation column was directly connected to the nebulizer of the ICP-MS using an ethylenetetra-fluoroethylene tube of 0.3 mm i.d.

Stock standard solutions of sodium arsenite, sodium arsenate, MMA, DMA and AsBe were prepared by dissolving each compound in ultra-pure water at a concentration of 100 mg As/l. The final diluted aqueous standard solution (100 µg/l) was prepared from stock standard solution just before use. To obtain precise measurements, 1 mg/l of germanium solution was used as the internal standard for ICP-MS. The ICP-MS detection mass was set to m/z 75 ($^{75}As^+$), m/z 72($^{72}Ge^+$), and m/z 77($^{40}Ar^{37}Cl$). This method was linear in the range 0.001–10 mg As/l, and the reproducibility for 0.01 mg As/l standard arsenic compound was about 2%.

Preparation of commercial Hijiki food

A 5 g portion of whole liquid mixture of the food was ground in a Polytron® homogenizer (PT-120, KINEMATICA), mixed with 10 ml 20% ethanol, left to stand overnight at room temperature and centrifuged at 3000 rpm for 15 min. After separation of the supernatant, the residue was again extracted with 10 ml 20% ethanol and centrifuged, and the supernatant combined with the first extract. This procedure was repeated three more times. After all five supernatants were combined and filtered through a 0.45 µm PTFE membrane (Millex-FH, Millipore Corp., MA, USA), the filtrate was diluted 10-fold with super-pure water, and the concentrations of arsenic species were determined using HPLC-ICP-MS as mentioned above. For calculation of recovery rates, the Hijiki sample was mixed with 100 ng As/g of each species of arsenic.

Total arsenic was determined using ICP-MS with the Dynamic Reaction Cell (ELAN DRCII, PerkinElmer SCIEX, Canada) after wet digestion using the Microwave Digestion System (MCS 950, PROLAB, USA), according to the method of Mochizuki *et al*.[35] One gram of homogenized food and 10 ml of 68% HNO_3 were mixed, digested by MCS 950, diluted 10-fold with pure water, and measured for total arsenic concentration by ELAN DRC II ICP-MS.

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Urinary arsenic analysis

The times and sample volumes of urination were recorded, and samples stored in sealed plastic tubes at 4 °C in a refrigerator until analysis, which was performed within one week. The samples were cleared and not filtered before analysis. Arsenic species in urine were stable under the conditions described above.[36,37]

Creatinine in urine was analyzed photometrically using creatinase and *N*-(3-sulfpuropyl)-3-methoxy-5-methylaniline by a commercial kit (Pure Auto CRE-N, Daiichi Pure Chemicals Co. Ltd, Tokyo, Japan). Urine samples were diluted 10-fold with super-pure water and analyzed by HPLC-ICP-MS as mentioned above.

For validation of urinary inorganic and methylated arsenic analysis, we have been participating every year in the Intercomparison Programme for toxicological analyses in biological materials organized by the German External Quality Assessment Scheme (G-EQUAS). We analyzed two types of reference urine certified for AsIII, AsV, MMA and DMA concentrations, by participating in this program in 2005.

RESULTS

The accuracy of the present analytical procedure was tested by analyzing NIES CRM no. 18, which is certified for DMA, AsBe, and total arsenic. The values were within the allowable errors for certified values, as shown in Table 1. In addition, the results of G-EQUAS-36 in 2005 for analysis of reference samples of human urine, which is certified for AsIII, AsV, MMA and DMA, were within the allowable ranges for certified values, as shown in Table 2. Both determinations were performed by calculation of peaks on the chromatograms obtained by HPLC-ICP-MS analysis.

Prior to refraining from eating seafood, the volunteer's total urinary arsenic concentration was 90.5 μg/g creatinine, and the major arsenic species were AsBe and DMA, as shown in Fig. 1. Five unidentified arsenic peaks were detected. After three months' restriction of ingestion of seafood, total urinary arsenic concentration had decreased to 11.3 μg/g creatinine, and one unidentified arsenic was found.

In 330 g of whole processed Hijiki food, 629 μg of AsV, 196 μg of AsIII, 35 μg of DMA and 12 μg of unidentified arsenic were detected. The sum of the detected arsenic on the chromatogram was 869 μg. Total arsenic determined after microdigestion of homogenized Hijiki food was 1253 μg. The chromatogram of the arsenic compounds in Hijiki is shown in Fig. 2. The recovery rates of AsV, AsIII and DMA added to the Hijiki food were 104.2, 95.0 and 100.7%, respectively.

Table 1. Arsenic analysis of reference urine of NIES CRM no. 18

Arsenic speciation	Our results (n = 5) (mean ± SD), μg As/l	Reference value (tolerance range), μg As/l
Total arsenic	131.5 ± 1.2	137 ± 11
Arsenobetain	70.1 ± 1.0	69 ± 12
DMA	37.2 ± 0.5	36 ± 9

Analysis was performed by HPLC-ICP-MS.

Table 2. Results of G-EQUAS-36 in 2005 for analysis of reference samples of human urine

Sample	Arsenic speciation	Our results ($n = 5$) (mean ± SD), μg As/l	Reference value (tolerance range), μg As/l
A	AsIII	3.7 ± 0.2	5.1 (3.0–7.1)
	AsV	6.0 ± 0.6	5.7 (3.7–7.6)
	MMA	5.6 ± 0.3	5.9 (4.8–6.9)
	DMA	22.6 ± 0.7	23.7 (17.6–29.7)
B	AsIII	9.3 ± 0.3	10.6 (8.6–12.5)
	AsV	9.2 ± 0.4	10.1 (5.7–14.4)
	MMA	10.5 ± 0.5	10.5 (9.0–11.9)
	DMA	30.7 ± 0.6	33.1 (25.0–41.1)

Analysis was performed by HPLC-ICP-MS.

Detected peak patterns differed among the times of urine sampling, as shown in Fig. 3. A maximum of nine unidentified arsenic peaks were detected, and the retention time of the peak matched with TMAO was detected at trace level 15.3 to 26.2 h after ingestion.

The time courses of urinary arsenic concentrations after ingestion are shown in Fig. 4. Maximum concentrations of AsV, AsIII, MMA and DMA were found at 4, 6.5, 13 and 17.5 h after ingestion, respectively. AsBe concentration was very low, and almost constant throughout the observation period. By 50 h after ingestion, AsV, AsIII and MMA concentrations had returned to baseline levels, although DMA concentration had not.

Changes in rates of excretion (μg As/h) of arsenic compounds are shown in Fig. 5. The rate of excretion of AsV was highest in the first voided urine, and decreased with time. The highest rate of excretion of AsIII was found for the second urination. In the first and second voided urines, the largest proportion of excreted arsenic was AsIII, which accounted for 40.7 and 40.6% of total arsenic, respectively. After the third urination, the major part of arsenic excreted was DMA, which gradually increased in proportion during the observation period.

The total amounts of AsV, AsIII, MMA, DMA, AsBe and unidentified arsenic excreted in urine over 50 h were 11.2, 31.8, 40.9, 104.0, 6.4 and 45.5μg, respectively. Their combined total arsenic was 239.8 μg, and 27.6% of total ingested arsenic.

DISCUSSION

The results of analysis of reference urine in this study validated our urinary arsenic analysis by HPLC-ICP-MS not

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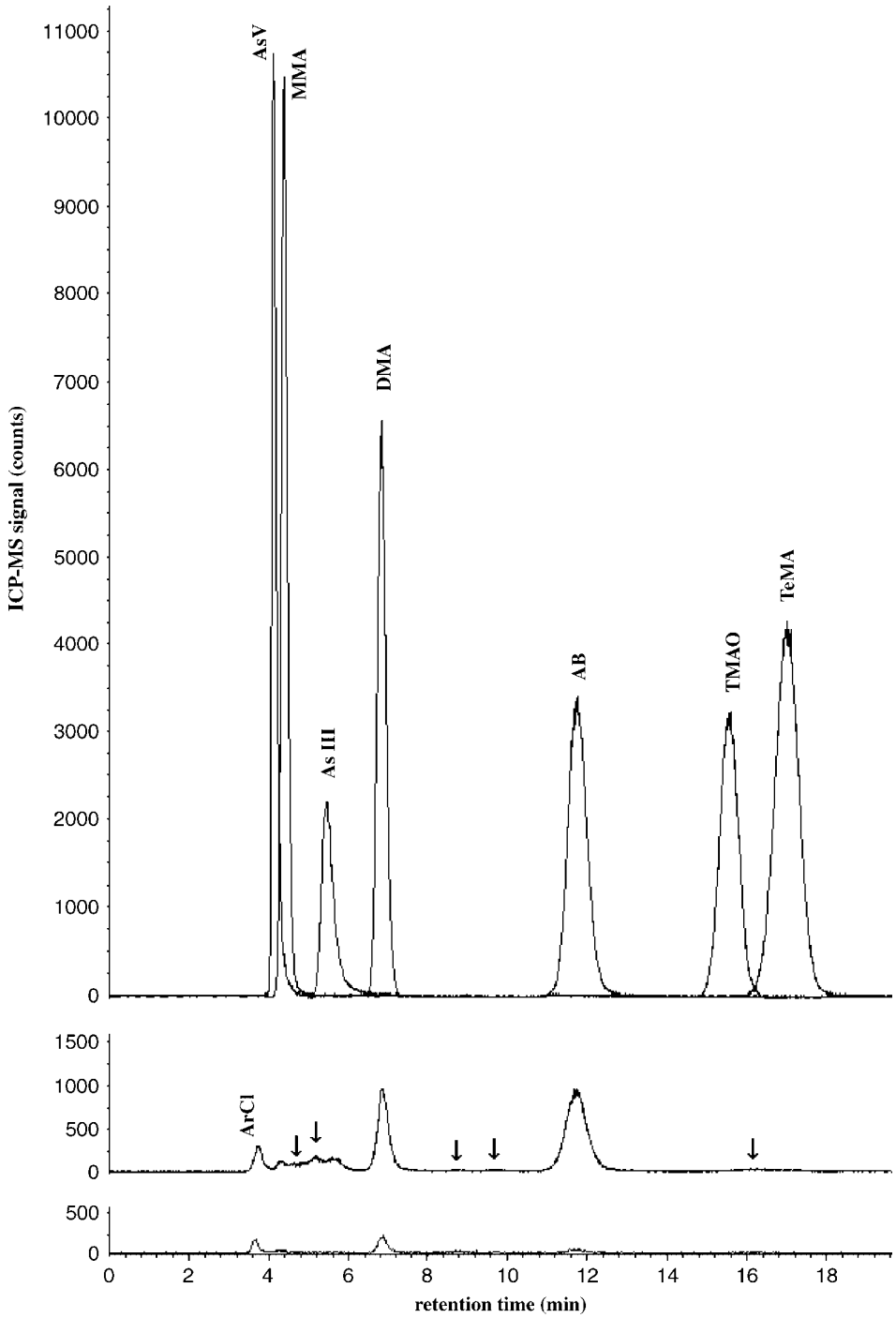

Figure 1. Chromatograms of standard and urinary arsenics: upper, middle, and lower chromatograms show standard solution and findings obtained before and after restriction of seafood ingestion, respectively.

only for arsenic speciation analysis but also total arsenic measurement.

There have been few reports on AsV intake and metabolism in humans. Many reports have been published regarding arsenic exposure to seafood ingestion, but none has revealed significant iAs exposure from seafood ingestion.[14,38,39] Our volunteer ate commercial processed Hijiki food in which Hijiki and beans were almost equal in content. In our speciation analysis, almost all of the arsenic in this food was found to be iAs, and AsV was the major arsenic species. Neither MMA nor AsBe was found, and only small amounts of DMA were. Raab *et al*.[32] reported that the major arsenic species in Hijiki was AsV, followed by arsenosugars, that DMA was a minor constituent, and that the concentrations

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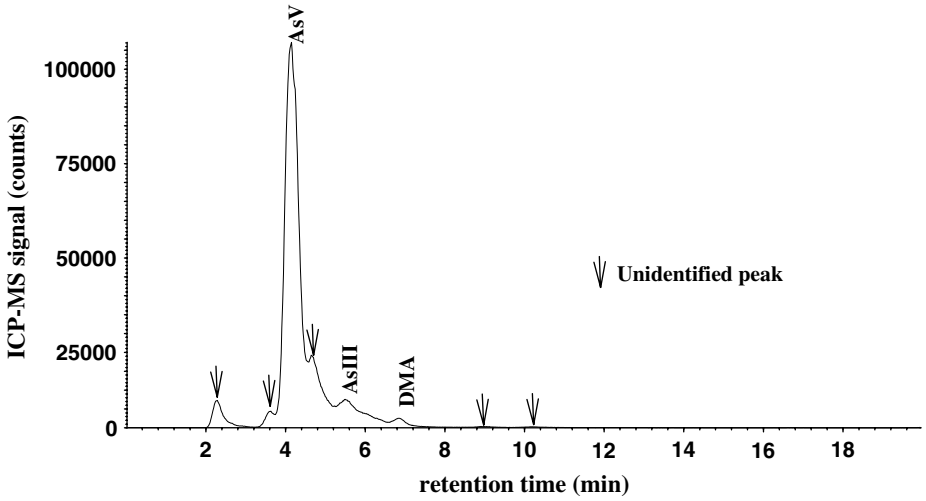

Figure 2. Chromatogram of arsenics in processed Hijiki food.

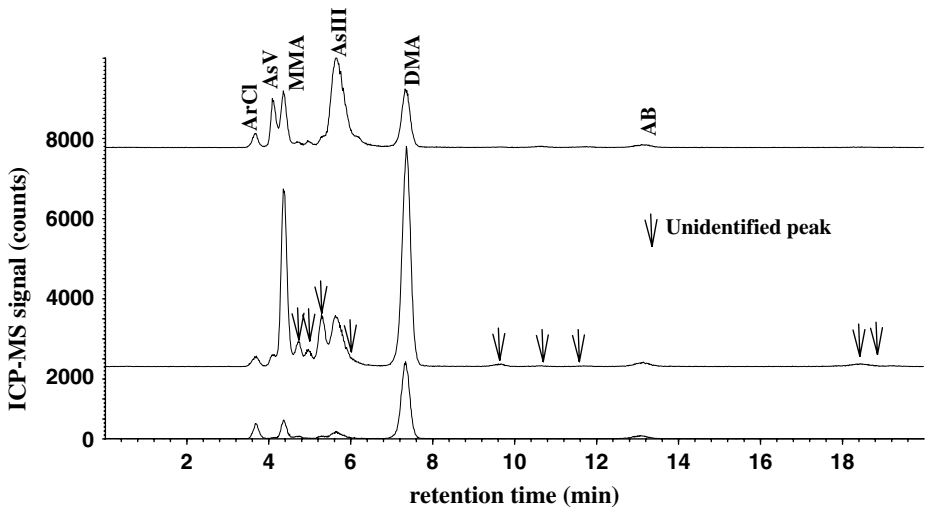

Figure 3. Chromatograms of urinary arsenics: upper, middle and lower chromatograms show findings obtained 6.3, 15.3 and 49.8 h after Hijiki ingestion, respectively.

of AsIII, AsBe, and arsenocholine were at or below levels of detection. Our results are consistent with their report, except for AsIII concentration. The effect of heat-processing (steaming or boiling) on arsenic species and their contents in Hijiki were studied by Kato *et al*. (unpublished data). Their preliminary results indicated that much of arsenic in Hijiki, particularly organic arsenics such as arsenosugars, were decomposed when whole Hijiki plants underwent heat-processing. Accordingly, the constitution of arsenic in Hijiki after heat-processing appears to vary. Thus, the high AsIII concentration in our Hijiki food could have been produced by heat-processing.

In general, seafood contains large amounts of organic arsenics such as AsBe, arsenocholine and arsenosugars, while content of iAs is below 1–2% of total arsenic.[13,40] Therefore, this constituent of the processed Hijiki food we used differs significantly from that in other seafoods.

Before our subject refrained from eating seafood, his urinary AsBe and DMA levels were very high but equal to those of Japanese individuals who habitually consume seafood.[5] Three months of abstaining from seafood intake decreased his baseline urinary arsenic level to 11.8 μg/g creatinine, which is within the range of non-smokers who have refrained from eating seafood.[38]

After Hijiki ingestion, arsenic compounds were excreted in the order AsV, AsIII, MMA and DMA, as shown in Fig. 4. This order is consistent with the metabolic pathway of iAs described in previous reports.[4,9] Also, many

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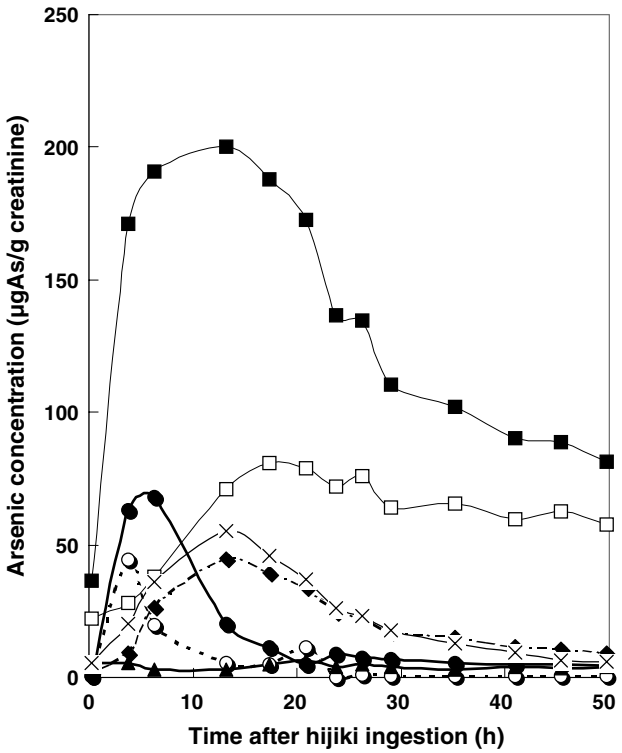

Figure 4. Urinary arsenic levels for 50 h after Hijiki ingestion. Symbols are as follows: ○, AsV; ●, AsIII; ♦, MMA; □, DMA; ▲, AsBe; ×, unidentified arsenic; ■, total arsenic concentration.

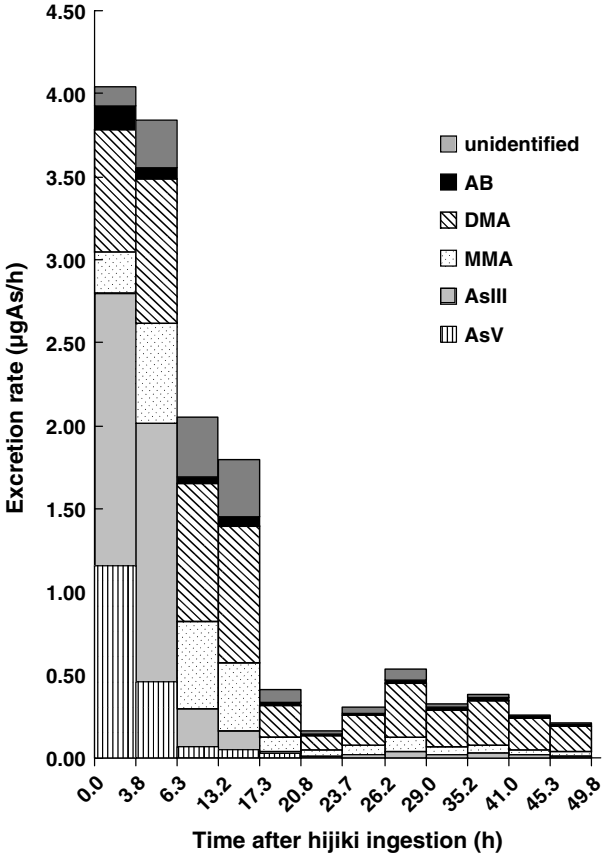

Figure 5. Time courses of rates of excretion of arsenic compounds.

unidentified arsenic peaks were detected, although each of their concentrations was trace level. Arsenosugar is a broad term for carbohydrate compounds containing arsenic, and 15 different arsenosugars have been identified in the marine environment.[29] Four kinds of arsenosugar were detected in Hijiki.[32] Ingested arsenosugars were quickly and almost completely metabolized to at least 12 arsenic products, some of which were thio-arsenicals in humans.[26,28] Although arsenosugars are one of the major constituents of Hijiki, they are easily transformed to various arsenic compounds not only by heat-processing but by metabolic change. In our HPLC-ICP-MS food analysis, five unidentified peaks were found, as shown in Fig. 2, but on analysis of urine many more unidentified peaks were detected, as shown in Fig. 3. Since almost half of the Hijili food ingested consisted of beans, we chose 20% ethanol to extract the arsenic in Hijiki and this solvent yielded complete recovery of the added arsenics. However, the sum of arsenic determined by chromatographic analysis was 69% of the total arsenic obtained by acid-digestion and DRC-ICP-MS measurement. It thus appeared that some organic arsenic compounds in Hijiki food might not be extracted with 20% ethanol, but that the ingested compounds were excreted into urine as unidentified peaks following metabolism. Since we do not have standard materials for arsenosugars, we cannot clearly demonstrate the unidentified arsenic peaks to be those of arsenosugars; however, these peaks are probably related to arsenosugars such as products of decomposition or metabolites.[25,26,28]

Rapid clearance of AsV and AsIII was found. On the other hand, excretion of MMA and DMA gradually increased and then slowly decreased. After 50 h, DMA level had not yet returned to the level before ingestion. AsV, AsIII, MMA and DMA accounted for 6.1, 17.1, 21.9 and 55.0% of excreted arsenic, respectively.

Inorganic arsenic is easily absorbed in the gastrointestinal tract and is eliminated primarily via the kidney in humans.[1,4] About 70–90% of a single dose of dissolved iAs was absorbed from the gastrointestinal tract of humans and 45–75% of the dose was excreted in urine within 4–5 days.[1,41,42] Over a 50 h observation period, 28% of the ingested arsenic was excreted in urine in our study. It is reported that 35% of ingested arsenic was excreted in urine during the 48 h period following intake of 500 μg of AsIII in water.[43,44] Also 38% of the ingested 650–760 μg of total arsenic was excreted in urine after drinking arsenate-rich seaweed extract solution.[45] The total rate of arsenic excretion in our study was somewhat lower than in these previous reports. Since the major arsenic species in Hijiki is AsV, the major proportion of ingested iAs from Hijiki may be quickly absorbed and excreted mainly into urine. Since we did not measure arsenic in feces, we

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could not estimate how much iAs was excreted over the 50 h period following ingestion. It has been reported that the bioavailability of ingested iAs varies depending on the matrix in which it is ingested, the solubility of the arsenical compound itself, and the presence of other food constituents and nutrients in the gastrointestinal tract.[1] For example, meals that are rich in fiber markedly decrease the absorption of AsV from the gastrointestinal tract.[46] Since Hijiki has large amounts of dietary fiber and the beans co-ingested with it were also rich in dietary fiber, absorption of iAs from the gastrointestinal tract may have been significantly suppressed.

The results of this study indicate that Hijiki ingestion can be considered equivalent to AsIII intake from polluted water. The urinary arsenic level of our volunteer was close to that of individuals with hyperkeratosis and hyperpigmentation in regions endemic for arsenic poisoning.[47] Since the quantity of Hijiki the volunteer ingested was equivalent to that in eight servings, one serving contained 102 μg of iAs. A dose-dependent increase in prevalence of hyperpigmentation was observed among individuals exposed to arsenic by drinking iAs at levels of <50, 50–99 and 100–149 μg/l water; disease occurred in 12/3467, 17/771 and 46/587 individuals, respectively.[48] In one study, the multivariate-adjusted relative risk of developing transitional cell carcinoma was 8.2 (95% CI 0.7–99.1) for arsenic concentrations of 50.1–100 μg/l compared with the reference level of ≤10.0 μg/l.[49]

Our study thus demonstrated that Hijiki ingestion is equivalent to iAs exposure, and that levels of iAs in Hijiki are similar to those in polluted drinking water. These findings suggest that, although no significant health risk of ingestion of seaweed has been reported, repeated daily ingestion of Hijiki as a side dish might increase the risk of arsenic poisoning.

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