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Effects of the fractionated components of the seaweed *Hizikia fusiforme* Okam. on arsenic metabolism in rats administered a large dose of arsenate†

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To investigate factors in the seaweed Hijiki affecting arsenic metabolism in rats, we extracted pulverized Hijiki samples with hot 0.1 M NaOH, and neutralized the extract with HCl. After centrifugation at 10 000 rpm for 30 min, the supernatants and the residues were lyophilized, mixed with cellulose, and added to the standard diet for rats. The rats were divided into three groups as follows: (1) group C, fed the standard diet, (2) group H-sup, fed a diet containing the extracted soluble fraction, and (3) group H-rsd, fed a diet containing the residues. After feeding the diets for 12 days, the respective groups were divided into two sub-groups; one of them was administered a large oral single dose (40% of the LD_{50}) of an aqueous solution of sodium arsenate, $Na_2AsHO_4{\cdot}7H_2O$, 24 h before sacrifice. Samples of urine were gathered before and after the administration of arsenate. Arsenic compounds in the urine and serum were analyzed by high-performance liquid chromatography–inductively coupled plasma mass spectrometry system. The patterns of the arsenic compounds suggested that some Hijiki components accelerated arsenic metabolism, e.g. methylation, in rats. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: ***Hizikia fusiforme*** **Okam.; alkaline extracts; rats; sodium arsenate; ICP-MS; arsenic compounds; serum; urine**

INTRODUCTION

In the seaweed family *Phaeophyceae*, which includes Hijiki, arsenic has often been found at relatively high levels.[1,2] The contents and distribution of arsenic in the individual plants were not uniform,[3] and some amounts of arsenic could be lost during the harvesting and manufacturing processes of Hijiki products. Thus, commercial products of Hijiki often showed different levels of arsenic according to the lot.[4,5] Japanese people traditionally consume large amounts of Hijiki as a major seaweed foodstuff,[6] as it constitutes a good source for minerals and beneficial dietary fiber.[7,8] We have fed rats with a Hijiki diet to investigate the effects on arsenic distribution in their organs,[4,5] and the results suggested that there was an acceleration of arsenic metabolism, including detoxification.[9]

In the present study, we fed rats with diets containing soluble and insoluble fractions of Hijiki plants to examine its effects on the amounts of various arsenic metabolites excreted in the urine, using inductively coupled plasma mass spectrometry (ICP-MS) analysis.

EXPERIMENTAL

Hijiki plants

Commercial products (samples) of Hijiki, *Hizikia fusiforme* Okam., harvested along the seashore of the Tsushima Archipelago, Japan, were generously donated by the Tsushima Archipelago's Third Sectional Hijiki Processing Company, Tsushima, Nagasaki Prefecture, Japan.

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Fractionation of Hijiki plants

Commercial products of dried Hijiki samples were soaked in sufficient distilled water for 30 min and washed with water three times. They were dried at room temperature and pulverized with a pulverizing machine. To the pulverized Hijiki was added 15 volumes of 0.1 M NaOH and the mixture was stirred at 80 to 90 °C for 20 min. After cooling to room temperature and neutralization with 0.1 M HCl, the mixture was centrifuged at 12000 rpm for 30 min. The supernatant was lyophilized and designated as fraction **H-sup**. The residue was dried at 35 °C and designated as fraction **H-rsd**.

Experimental diet compositions

The experimental diet of 100 g was composed of 63 g corn starch, 20 g casein, 5 g corn oil, 5 g of a mineral mixture, 2 g of a vitamin mixture and 5 g cellulose powder for the **control** group, designated as group **C**. For the **H-sup** diet group, the cellulose was replaced with 3.5 g of cellulose plus 1.5 g of fraction **H-sup**; for the **H-rsd** diet group, the cellulose was replaced with 1.5 g of cellulose plus 3.5 g of fraction **H-rsd**. The composition of 100 g of the vitamin mixture was: vitamin A acetate, 50000 IU; vitamin D_3, 10000 IU; vitamin B_1·HCl, 120 mg; vitamin B_2, 400 mg; vitamin B_6·HCl, 80.0 mg; vitamin B_{12}, 0.05 mg; vitamin C, 3,000 mg; vitamin E acetate, 500 mg; vitamin K_3, 520 mg; D-biotin, 2 mg; folic acid, 20 mg; calcium pantothenate, 500 mg; *p*-aminobenzoic acid, 500 mg; nicotinic acid, 600 mg; inositol, 600 mg; choline chloride, 20000 mg; cellulose, 73.05 g. The mineral mixture consisted of $CaHPO_4·2H_2O$, 14.56%; KH_2PO_4, 25.72%; $NaH_2PO_4·H_2O$, 9.35%; NaCl, 4.66%; calcium lactate, 35.09%; iron citrate, 3.18%; $MgSo_4·3H_2O$, 7.17%; $ZnCO_3$, 0.11%; $MnSO_4$, 0.14%; $CuSO_4$, 0.03%; KI, 0.01%.

Animals

Three week-old male Sprague-Dawley rats (about 50 g in body weight) were used.

Feeding conditions

Rats were initially fed on the MF chow of Oriental Yeast Co., Ltd, for 7 days, and separated into three groups of nine rats each. Individual rats were kept separately in a stainless metabolic cage and fed with the respective experimental diets *ad libitum* for 12 days. On day 13, six rats of each group were starved for 24 h and administered arsenate (see below), and the individual groups were designated as **C-As, H-sup-As**, and **H-rsd-As**. Then, they were put back on the experimental diet for further 24 h, and sacrificed to obtain blood samples.

To the remaining three rats of each group, the same amount of distilled water was administered instead of the arsenate solution, and the individual groups were designated as **C-n, H-sup-n** and **H-rsd-n**.

The individual food intake and body weight were measured every day.

Arsenate administration

On day 13, 0.4 ml of sodium arsenate ($Na_2AsHO_4·7H_2O$; 14 mg arsenic) aqueos solution per kilogram of body weight was administered through a stomach tube, the dose corresponding to 40% of the LD_{50} of rats.

Sampling of the urine

Urine was collected for 24 h before starvation (designated as **Pre24h**), and for the first 6 h (designated as **0-6h**) and following 18 h (designated as **6-24h**) of refeeding. Portions of the urine samples, stored in a deep freezer, were used for arsenic determination.

Sampling of the blood and serum

The blood, collected from the rats 24 h after the arsenate administration, was separated into blood cells and serum by centrifugation. The serum samples were stored in a deep freezer until analysis.

Analysis of arsenic compounds by high-performance liquid chromatography (HPLC) ICP-MS

Arsenic compounds were analyzed with an ICP-MS instrument equipped with HPLC columns of LC600VS (GL Sciences, Japan). Samples were applied to the Intersil AS (3 μm, 2.1 × 150 mm, 2.1 × 250 mm, GL Sciences, Japan) column and eluted with a solvent of 10 mM sodium 1-butane sulfonate, 4 mM tetramethylammonium hydroxide, 4 mM malonic acid, 0.05% methanol (adjusted to pH 3.0 with HNO_3) at 45 °C. Arsenic compounds in the eluates were

Table 1. Volume of urine

Diet group[a,b]	Urine samples[c] (*ml*)		
	Pre-24h	**0-6h**	**6-24h**
C-n[d]	10.4 ± 2.4	0.7 ± 0.2	8.3 ± 0.9
H-sup-n[d]	7.7 ± 0.5	0.9 ± 0.0	8.5 ± 0.9
H-rsd-n[d]	10.3 ± 1.3	1.0 ± 0.2	10.8 ± 0.1
C-As[e]	11.0 ± 1.5	0.3 ± 0.1	2.2 ± 0.5
H-sup-As[e]	7.6 ± 1.3	0.8 ± 0.5	2.4 ± 0.8
H-rsd-As[e]	9.0 ± 1.4	0.2 ± 0.1	2.0 ± 0.5

[a] The rats were fed with either the control diet (designated as **C**), Hijiki supernatant diet (designated as **H-sup**), or Hijiki residue diet (designated as **H-rsd**).
[b] The arsenate-administered group is indicated with the suffix **-As** and the untreated group with **-n**.
[c] Urine samples were collected for 24 h before (designated as **Pre-24h**) and after (designated as **0-6h** and **6-24h**) 24 h starvation. The arsenate was administered after the 24 h starvation, and then urine samples were collected for the first 6 h (designated as **0-6h**) and succeeding 18 h (designated as **6-24h**).
[d] Values expressed as mean ± SE of three rats.
[e] Values expressed as mean ± SE of six rats.

Table 2. Composition of arsenic compounds in urine[a]

	C-As group			**H-sup-As** group			**H-rsd-As** group		
	Concentration (ppb)	Total amount per rat (μg)	Composition (%)	Concentration (ppb)	Total amount per rat (μg)	Composition (%)	Concentration (ppb)	Total amount per rat (μg)	Composition (%)
Pre-24h									
Arsenate	114	0.83	39.2	53	0.30	5.5	201	1.10	35.7
Arsenite	131	0.96	45.3	134	0.75	13.7	104	0.52	16.9
Methylarsonic acid			0.0			0.0	153	0.54	17.5
Dimethylarsenic acid	43	0.33	15.6	768	3.75	68.4	70	0.24	7.8
Trimethylarsine oxide			0.0	26	0.12	2.2			0.0
Tetramethylarsonium salt			0.0	50	0.24	4.4			0.0
Arsenobetaine			0.0	48	0.23	4.2	162	0.68	22.1
Arsenocholine			0.0	19	0.09	1.6			0.0
Sum		2.12	100.0		5.48	100.0		3.08	100.0
0-6h									
Arsenate	5686	1.78	18.7	2593	1.32	31.1	3018	0.60	8.7
Arsenite	10592	2.09	22.0	529	0.56	13.2	5614	1.41	20.5
Methylarsonic acid	10626	2.98	31.3	3666	0.73	17.2	8205	2.33	33.8
Dimethylarsenic acid	8824	2.66	28.0	5677	1.60	37.7	8895	2.55	37.0
Trimethylarsine oxide			0.0	87	0.02	0.5	17	0.00	0.0
Tetramethylarsonium salt			0.0	41	0.01	0.2			0.0
Arsenobetaine			0.0			0.0			0.0
Arsenocholine			0.0	16	0.00	0.0	15	0.00	0.0
Sum		9.51	100.0		4.24	99.9		6.89	100.0
6-24h									
Arsenate	6108	6.96	27.5	2663	3.59	64.8	6165	13.79	21.7
Arsenite	9429	11.84	46.9	1683	1.42	25.6			0.0
Methylarsonic acid	3169	2.98	11.8			0.0	11342	25.50	40.2
Dimethylarsenic acid	4205	3.15	12.5	604	0.53	9.6	10243	23.37	36.8
Trimethylarsine oxide	38	0.03	0.1			0.0	48	0.11	0.2
Tetramethylarsonium salt	38	0.03	0.1			0.0	429	0.68	1.1
Arsenobetaine	177	0.28	1.1			0.0			0.0
Arsenocholine			0.0			0.0			0.0
Sum		25.27	100.0		5.54	100.0		63.45	100.0

[a] Experimental conditions and designations are as described in Table 1 and the text.

Table 3. Composition[a] of arsenic compounds in serum

	C-As group[b]		**H-sup-As** group[b]		**H-rsd-As** group[b]	
	Total amount per rat (μg)	Composition (%)	Total amount per rat (μg)	Composition (%)	Total amount per rat (μg)	Composition (%)
Arsenate	4.71	51.8	5.45	54.1	3.46	48.0
Arsenite	1.21	13.3	0.68	6.7	0.91	12.6
Methylarsonic acid	0.48	5.3	0.71	7.0	0.39	5.4
Dimethylarsenic acid	2.60	28.6	3.05	30.3	2.02	28.0
Trimethylarsine oxide	0.00	0.0	0.00	0.0	0.15	2.1
Tetramethylarsonium salt	0.00	0.0	0.00	0.0	0.09	1.2
Arsenobetaine	0.09	1.0	0.12	1.2	0.12	1.7
Arsenocholine	0.00	0.0	0.07	0.7	0.07	1.0
Sum	9.09	100.00	10.08	100.00	7.21	100.00

[a] Average values of duplicate or triplicate analyses.
[b] Experimental conditions and designations are as described in Table 1 and the text.

monitored at *m/z* 75 with an SPQ 9200 ICP mass spectrometer (Seiko Instrument Inc., Japan).

Authentic samples of arsenate, arsenite, methylarsonic acid, dimethylarsinic acid, arsenobetaine, trimethylarsine oxide, tetramethylarsonium salt, and arsenocholine were used for qualitative and quantitative calibration of the arsenic compounds in the samples.

Analytical details have been published previously.[10] Chromatograms and further analytical details are available from the authors on request or within Ref. 10.

RESULTS

Food intake and body weight gain

The daily food intake and body weight gain were constant during the experimental period before starvation, suggesting physiological constancy, as shown by the constant ratio of 0.4 of the body weight gain to the food intake in all the groups.

Volume of urine

The average volume of urine before starvation was 9.4 ml in 24 h (**Pre24h**). After arsenic administration, the urine volume was 0.4 ml for the first 6 h (**0-6h**), and 2.2 ml for the succeeding 18 h (**6-24h**), as shown in Table 1. The urine volumes did not differ significantly between the three diet groups.

Arsenic compositions of the urine

The amounts of arsenic compounds excreted in the urine are shown in Table 2. Before starvation, in the control group (**Pre-24h** of **C-As** group) the percentages of urinary arsenate, arsenite and dimethylarsenate were about 40%, 45%, and 16% respectively (Table 2). It is noticeable that, after arsenate administration, a higher ratio of methylarsonate was excreted into the urine.

In the **Pre-24h** columns of Table 2, the patterns of arsenic excretion were different between the groups of the supernatant (**H-sup**) and residues (**H-rsd**) of Hijiki plants, probably reflecting different types of arsenic compounds in both fractions. In the **H-rsd-As** group, the ratio of arsenite decreased and much higher amounts of methylarsonate and dimethylarsenate were excreted for 24 h after the arsenate administration. On the other hand, the **H-sup-As** group showed a lower amount of methylarsonate during the 24 h after the arsenate administration (the values of **0-6h** plus those of **6-24h**).

Arsenic compositions in the serum

The arsenic compositions in the serum are shown in Table 3. On comparison between the **C-As, H-sup-As** and **H-rsd-As** groups, the effects of the **H-rsd** fraction are seen in the values of slightly less arsenate and significant levels of both trimethylarsine oxide and tetramethylarsonium salt. However, the percentage compositions of arsenic compounds in the serum were not so different from each other between the groups, compared with those of the urinary arsenic compounds shown in Table 2.

In the serum of the **H-sup-n** and **H-rsd-n** group, arsenobetaine alone was mostly detected: 0.66 ng per rat in the **H-sup-n** group and 2.17 ng per rat in the **H-rsd-n** group.

DISCUSSION

The reduction of the urine volume in the arsenate-administration groups may indicate some physiological damage of the kidney by the large dose of arsenate. Although the patterns of percentage compositions of the arsenic compounds in the urine (Table 2) differ from those in the serum (Table 3), the patterns in the urine may more clearly reflect arsenic metabolism in the various organs than do those in the serum. In the serum, physiological homeostasis of blood may have made those patterns resemble each other between

the three groups, i.e. the **C-As**, **H-sup-As** and **H-rsd-As** groups.

The amounts of arsenic compounds in the urine before starvation (**Pre-24** in Table 2a–c) indicate the effects of the **H-rsd** and **H-sup** fractions on arsenic metabolism and/or the arsenic compounds derived from the Hijiki fractions.

After arsenate administration, the composition of arsenic compounds in the urine will reflect arsenic metabolism. The differences between the values of **Pre-24h** and those of **0-6h** plus **6-24h** will indicate the effects of the Hijiki plants on arsenic metabolism. Moreover, the two Hijiki fractions (**H-sup** and **H-rsd**) may have different effects on the accumulation of arsenic in the various organs, as well as on arsenic metabolism (Table 2).

These will be elucidated further by tracer experiments, but it is clear that the enzymic activities in the methylation process are affected by the Hijiki fractions. These data are interesting in view of the phenomena found in the altered rate of arsenic accumulation in the liver by the Hijiki diet,[9] the mechanism of which is under investigation.

CONCLUSION

The rats fed a diet of **H-rsd** showed a pattern of the composition of arsenic compounds in their serum and urine different from that in rats fed the **H-sup** diet or the control diet. This suggests the effectiveness of Hijiki plants, especially of the **H-rsd** fraction, on arsenic metabolism leading to arsenic detoxification, including methylation, in rats.

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