APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 8, 259–264 (1994)

Effects of Hijiki Feeding on Arsenic Distribution in Rats Administered Large Doses of Arsenate

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Male rats fed a diet of 5% Hijiki seaweed or 5% cellulose for two weeks were administered large doses of sodium arsenate (Na_2HAsO_4) at 7 mg As kg^{-1} of body weight, during two days. At 48 h following the last arsenic administration, selected organs were isolated and homogenized. Femur and feces were lyophilized; portions of them were irradiated with thermal neutrons in a research nuclear reactor and their arsenic concentrations were determined from the induced gamma radiation from ^{76}As. The greatest concentration of arsenic was detected in blood cells. A greater arsenic level was found in urine from the Hijiki diet group than in that of the cellulose diet group. The percentage distribution of arsenic in various organs indicated that the arsenic concentrated in blood cells 48 h after the arsenate administration, in comparison with the value 24 h after the arsenate administration [M. Katayama *et al.*, *Appl. Organomet. Chem.* 6 389 (1992)]. The Hijiki diet accelerated arsenic accumulation in blood cells and the femur more than the cellulose diet, and arsenic levels in other organs (liver, heart, lung and kidney) of the Hijiki diet group decreased faster than those from the cellulose diet group.

Keywords: Arsenic metabolism, rats, Hijiki diet, arsenic distribution, neutron activation analysis, blood cells, organs, sodium arsenate

INTRODUCTION

Hijiki (*Hizikia fusisorme* Okam.) is a seaweed used in traditional Japanese food and it is recognized as an effective dietary fiber[1] as well as a mineral-rich source.[2] However, Hijiki has often contained high levels of arsenic.[3,4] Thus, it is of interest to know how a Hijiki diet affects arsenic metabolism. In a previous paper, the effect of a Hijiki diet on arsenic distribution in various organs has been reported:[5] liver, heart, lung and kidney contained significantly higher concentrations of arsenic than those of a cellulose diet group 24 h after the arsenic administration. It was intended to discover how in the course of time, the arsenic distribution changes in the organs of rats fed a Hijiki diet after administration of a large dose of arsenate. In this study, arsenic distributions in some organs were measured 48 h after the administration of a large dose of arsenate. The results indicated arsenic being concentrated in the blood cells of the Hijiki diet group more rapidly in the course of time than that of the cellulose diet group, suggesting effects of Hijiki diet on arsenic metabolism.

EXPERIMENTAL

Animals

Sprague–Dawley male rats, six weeks old, were fed a laboratory chow for one week, and then divided into two groups. One group was fed a 5% Hijiki diet, and the other group was fed a 5% cellulose diet for two weeks until sacrifice. At 72 h and 48 h before sacrifice, arsenate was administered. Each group consisted of five or six rats and each determination of arsenic was performed in triplicate.

Diet compositions

The compositions of the diets were as follows:[5] corn starch, 63%; casein, 20%; corn oil, 5%; mineral mixture, 5%; vitamin mixture, 2%; cellulose or Hijiki, 5%.

The mineral mixture consisted of $CaH_4(PO_4)_2.H_2O$, 14.56%; KH_2PO_4, 25.72%; $NaH_2PO_4.H_2O$, 9.35%; NaCl, 4.66%; calcium

CCC 0268–2605/94/030259–06

Received 10 December 1993
Accepted 18 January 1994

lactate, 35.09%; ferric citrate, 3.18%; $MgSO_4.3H_2O$, 7.17%; $MnSO_4$, 0.12%; $CuSO_4$, 0.03% and KI, 0.01%.

The vitamin mixture, per 100 g, was composed of: vitamin A acetate, 5×10^4 IU; vitamin D_3, 10^4 IU; vitamin B_1.HCl, 120 mg; vitamin B_2, 400 mg; vitamin B_6.HCl, 80 mg; vitamin B_{12}, 0.05 mg; vitamin C, 3000 mg; vitamin E, 500 mg; vitamin K_3, 530 mg; biotin, 2 mg; folic acid, 20 mg; pantothenate, 500 mg; *p*-aminobenzoic acid, 500 mg; nicotinic acid, 600 mg; inositol, 600 mg; choline chloride, 2×10^4 mg; and cellulose, 73.05 g.

Hijiki (*Hizikia fusiforme* Okam.) was obtained commercially: the leaves of Hijiki harvested on the shore of Goto Islands had been separated from the stems, steamed and dried for the commercial product. These dried leaves of Hijiki were soaked in water for one night, excess water was allowed to drip away and the leaves were dried in air for the experimental diets. The experimental Hijiki sample was pulverized and mixed well with the diet components to produce a 5% Hijiki diet. As a control, a 5% cellulose diet was prepared, using pure cellulose powder instead of Hijiki.

Arsenic concentration in diet

Hijiki used for the preparation of the diet (grade 3) contained 33.8 ± 1.1 ppm of arsenic in total. Samples of the 5% Hijiki diet contained 1.98 ± 0.28 ppm arsenic and those of the 5% cellulose diet, trace amounts only. These were determined by neutron activation analysis.

The Hijiki diet group administered arsenate was designated as **H(3)-As**, and that without arsenate administration as **H(3)-n**. The number in parentheses (**3**) indicates the grade, and hence the arsenic level in the Hijiki. Grade 1 Hijiki, designated as **H(1)**,[5] contains a few parts per million of arsenic; grade 2, designated as **H(2)**,[5] 70 ppm of arsenic; grade 3, designated as **H(3)**, 33 ppm of arsenic. The cellulose diet group was designated as **C-As** (arsenate administered) or **C-n** (arsenate not administered).

Arsenic administration

Sodium arsenate (Na_2HAsO_4) was dissolved in water (0.7 mg As cm^{-3}) and administered. A dose of 7 mg As kg^{-1} body weight was administered (3.5 mg As kg^{-1}, once daily) by stomach tube for two days. The rats were sacrificed 48 h after the last arsenic administration. Some of the Hijiki-group rats were not given a dose of arsenic (see preceding section for details).

Treatment of samples

The body weight of rats of the Hijiki group was 389.1 ± 4.7 g and that of the cellulose group was 362.0 ± 7.9 g (mean ± SEM).

The anesthetized rats were sacrificed by taking blood from abdominal aorta 48 h after the last arsenate administration. Liver, heart, lung, kidney, testis, adipose tissue and muscle were removed and homogenized with nine volumes of water at ice temperature. Liver and heart had been previously perfused. Portions of the homogenates were spotted onto pieces of filter paper and dried. Femur was removed and lyophilized. Carcasses (excluding the above organs and tissues, skin and tail) were homogenized with a mincing machine and some portions of the homogenates were lyophilized. The samples were irradiated with slow neutrons. Blood was centrifuged at 2000*g* for 20 min at 4 °C. Some portions of blood cells and plasma were spotted onto pieces of filter paper for activation analysis. Hair was sampled for activation analysis.

Sampling of feces and urine

At 24 h (designated as Day 1) and 48 h (designated as Day 2) after the last administration of arsenic, the urine and feces were collected. The urine was concentrated in a rotary evaporator. Some portions were spotted onto pieces of filter paper for neutron activation analysis. The feces were lyophilized and pulverized. Some portions were irradiated with slow neutrons.

Arsenic determination by thermal neutron activation analysis

The samples, sealed individually in polyethylene bags, were placed in polyethylene Neuma capsules and irradiated by a flux of 10^{13} slow neutrons $cm^{-2}\,s^{-1}$ for 20 min in the nuclear reactor of the Research Reactor Institute, Kyoto University.

After 60 h cooling time, gamma radiation from ^{76}As was determined using a Ge/Li detector at 559.1 keV. Energy levels of ^{60}Co and ^{137}Cs were used for calibration. The amount of arsenic in the samples was determined by comparison with authentic arsenate standards, spotted onto pieces

Table 1 Concentration of arsenic in the various organs of rats fed a 5% Hijiki diet or a 5% cellulose diet[a] (ppm[b])

Diet	Group[c]	Blood cells	Liver	Heart	Lung	Kidney	Testis	Adipose tissue	Muscle	Femur
5% Hijiki	**H(3)-As**[d, e]	79.14 ± 4.52	2.35 ± 0.34	2.27 ± 0.20	5.13 ± 0.98	4.25 ± 1.04	Trace	Trace	Trace	1.52 ± 0.20
5% Cellulose	**C-As**[f]	51.78 ± 15.53	2.71 ± 0.73	1.47 ± 0.18	3.29 ± 0.63	3.78 ± 0.96	Trace	Trace	Trace	1.00 ± 0.08

[a] Rats, having been fed a 5% Hijiki or a 5% cellulose diet for two weeks, were administered 7 mg As kg^{-1} body weight for two days. At 48 h after the last administration, the respective organs were separated. Samples were determined by neutron activation analysis, as described in the text.
[b] Mean ± SEM.
[c] See section on 'Arsenic concentration in the diet' for an explanation of the group descriptions and control experiment details.
[d] This Hijiki sample contained 33.7 ppm of arsenic, defined as Grade 3.
[e] Six rats.
[f] Five rats.

Table 2 Distribution of arsenic in individual organs (μg)[a]

Diet	Group	Blood cells[b]	Liver	Heart	Lung	Kidney
5% Hijiki	**H(3)-As**[c]	1227.03 ± 83.88	37.89 ± 5.66	2.60 ± 0.29	7.55 ± 1.52	11.50 ± 2.50
5% Cellulose	**C-As**[d]	748.66 ± 236.42	44.31 ± 16.76	1.53 ± 0.18	4.66 ± 1.10	9.37 ± 2.73

Experimental conditions were as described in Table 1 and the text.
[a] Mean ± SEM, per rat. [b] Volume was 8% of the body weight. [c] Six rats. [d] Five rats

of filter paper, placed between every 10–20 specimens.

RESULTS

Concentration and quantity of arsenic in blood cells

Blood cells accumulated major amounts of arsenic (Table 1). Blood cells of the **H(3)-As** group showed 79 ppm of arsenic, which came from the Hijiki diet as well as from administered arsenate. The arsenic level of blood cells of the **C-As** group was 52 ppm, contributed mostly from the administered arsenate. The **C-n** group accumulated about 1 ppm of arsenic in blood cells since weaning and the **H(3)-n** group accumulated 26 ppm after feeding on the Hijiki diet alone. The amount of arsenic in the blood cells of the **H(3)-As** group was 1227 μg (average) in an individual rat and that of the **C-As** group 749 μg (Table 2).

Concentration of arsenic in the various organs of rats

The amount of arsenic was expressed in ppm (μg As g^{-1} of tissue wet weight) (Table 1). The arsenic levels of lung, kidney, liver, heart and femur of the **H(3)-As** group were 5 ppm, 4 ppm, 2 ppm, 2 ppm and 1.5 ppm, respectively. The arsenic levels of the lungs, heart and femur of the **C-As** diet group were significantly lower than those of **H(3)-As** group, being 3 ppm, 1.5 ppm and 1 ppm, respectively. The **C-n** group showed less than 1 ppm As in the lung and only trace amounts in other organs.

Distribution of arsenic in the various organs

Amounts of arsenic in the respective organs were calculated (Table 2). In the liver of the **H(3)-As** group, 38 μg of arsenate was accumulated and in that of the **C-As** group, 44 μg. In the kidney, lung and heart of the **H(3)-As** group, 12 μg, 8 μg and 3 μg of arsenic accumulated respectively and in those of **C-As** group, 9 μg, 5 μg and 2 μg, respectively.

Percentage distribution of arsenic in the various organs

The values shown in Table 3 for the arsenate-administered group were adjusted to compensate for the values of the group not administered arsenate. The arsenic contents in organs of the **H(3)-n** group were in part interpolated from the values of the **H(1)-n** and **H(2)-n** groups published previously.[5]

The percentage of administered arsenic (arsen-

Table 3 Percentage distribution of arsenic in the various organs and tissues (%)[a]

Time after arsenic administration (h)	Diet	Group	Blood cells[b]	Liver	Heart	Lung	Kidney
24	5% Hijiki	**H(1)-As**	6.47	1.48	0.17	0.21	0.27
24	5% Cellulose	**C-As**	6.79	0.67	0.05	0.19	0.19
48	5% Hijiki	**H(3)-As**	33.5	0.48	0	0	0.14
48	5% Cellulose	**C-As**	29.0	1.45	0.05	0.13	0.32

Experimental conditions and designations were as described in Table 1 and the text.
[a] The percentage was expressed on the basis of the amount of arsenic administered.
[b] The blood volume was estimated.
[c] Data from Ref. 5.

Table 4 Concentration and quantity of arsenic in carcasses and hair

Diet	Group	Concentration (ppm)[a]		Quantity in an individual carcass (μg)[b]
		Carcass	Hair	
5% Hijiki	**H(3)-As**[c]	2.02 ± 0.17	Trace	134.0 ± 12.5
5% Cellulose	**C-As**[d]	1.84 ± 0.30	Trace	118.9 ± 20.5

Experimental conditions were as described in Table 1 and the text.
[a] Mean ± SEM.
[b] Mean ± SEM in a rat, based on values for individual rats.
[c] Six rats.
[d] Five rats.

ate) that was retained was 33.5% in the blood cells of the **H(3)-As** group and 29.0% in those of the **C-As** group 48 h after the last administration of arsenate; 24 h after arsenate administration,[5] the percentage in blood cells of the **H-As** group was 6.5% and that of the **C-As** group was 6.8%.

At 48 h after arsenate administration, in the liver and kidney of the **H(3)-As** group the percentage distribution was 0.48% and 0.14%, respectively. In the heart and lung of the **H(3)-As** group, arsenic distribution was very small. In comparison with previous results at 24 h after arsenate administration,[5] the respective values at 48 h for the various organs of the **H-As** group decreased, except for blood cells and femur.

These results indicate that the Hijiki diet is effective in the acceleration of arsenic accumulation in blood cells and femur, and in decreasing the arsenic levels in the other organs.

Concentration of arsenic in carcasses and hair of rats

Arsenic concentration in the carcasses of the **H(3)-As** group was 2.0 ppm and that of the **C-As** group, 1.8 ppm, but those values are not significantly different from each other. The total amount of arsenic in the carcasses of the **H(3)-As** group was 134 μg, and that of the **C-As** group was 119 μg (Table 4). Arsenic was hardly detected in the hair of either group (Table 4).

Table 5 Quantity of arsenic in urine (μg As day^{-1})[a]

Diet	Group	Day 1 urine	Day 2 urine
5% Hijiki (Grade 3)	**H(3)-As**[b]	11.05 ± 2.19	5.66 ± 0.82
5% Cellulose	**C-As**[c]	5.51 ± 1.13	6.20 ± 1.02

Experimental conditions were as described in Table 1 and the text.
[a] Mean ± SEM of a rat. [b] Six rats. [c] Five rats.

Quantity of arsenic in urine

In the urine samples from Day 1, about twice as much arsenic was found in the **H(3)-As** group compared with that in the **C-As** group (Table 5). This suggests enhancement of arsenic excretion by Hijiki.

Quantity of arsenic in feces and contents of large intestine, small intestine and stomach

The group fed the Hijiki diet did not show any detectable amounts of arsenic in the stomach contents 48 h after the last administration, although a few rats of the cellulose diet group indicated some arsenic in the stomach contents (Table 6). Between the **H(3)-As** and the **C-As** group, a different pattern of arsenic distribution was observed in the Day 1 feces, Day 2 feces, content of the large intestine, content of the small intestine and content of stomach. However, the total amount of arsenic in the feces and contents in the digestive tracts were not different between the two groups. These results show that the content in the digestive tract of the **H(3)-As** group transits faster than those of the **C-As** group.

Apparent absorption of arsenic

The summation of the arsenic content in the feces and the content of the digestive tract (large intestine, small intestine, and stomach) corresponds to the quantity of arsenic not retained in the body. Thus, 90% of the apparent absorption occurred after arsenate administration, in the case of both groups.

Table 6 Arsenic distribution in feces and in digestive tract contents (μg per individual rat)[a]

		Feces[b]		Contents of[c]		
Diet	Group	Day 1	Day 2	Large intestine	Small intestine	Stomach
5% Hijiki	H(3)-AS[d]	49.25 ± 8.66	128.01 ± 19.51	24.70 ± 5.50	—[f]	Not detected
5% Cellulose	C-As[e]	9.80 ± 3.03	129.08 ± 26.27	42.16 ± 7.12	—[g]	Trace

Experimental conditions were as described in Table 1.
[a] Mean ± SEM.
[b] Collected 24 h ('Day 1') and 48 h ('Day 2') after the last arsenic administration.
[c] 48 h after the last arsenic administration.
[d] Six rats, except large-intestine content (five rats).
[e] Five rats, except feces, Day 1 (four rats).
[f] One rat, with a lower amount of arsenic in Day 2 feces, showed 5.78 μg of arsenic in the contents of the small intestine. Another rat did not show any detectable amount of arsenic.
[g] One rat, with a lower level of arsenic in Day 2 feces, showed 4.72 μg of arsenic in the contents of the small intestine. Another rat did not show any detectable amount of arsenic.

DISCUSSION

As shown in the Tables, the rats accumulated the majority of the arsenic in their blood cells. Arsenic concentrations in the blood increased with age,[6] and even those of weaning rats were over 100 times higher than those of rabbits of the corresponding age. The high levels of arsenic in red blood cells have been attributed to the high activity of glutathione reductase, an enzyme which is not inhibited by arsineoxide. The probable position of the arsonous thioester group on hemoglobin does not affect its oxygen binding properties[7]. Further investigations concerning these aspects are taking place.

It is interesting that, in the course of time, a Hijiki diet accelerated an increase in arsenic concentrations in the blood cells and the femur also (Table 3). Although the percentage composition of arsenic distribution in the various organs of the rats was not the same as that of mice,[8] our preliminary results with mice suggest that a Hijiki diet also has some effect on arsenic metabolism in the body.

As suggested in Table 6, a Hijiki diet promoted faster transit of the contents in the digestive tract. This is a typical effective role of dietary fibers. Moreover, a Hijiki diet seems to have another kind of role also as demonstrated in our experiments on cholesterol metabolism with cecectomized rats (M. Katayama, Y. Sugawa-Katayama and K. Otsuki in preparation; even cecectomized rats of the Hijiki diet group showed significantly lower total cholesterol level in serum than those of cellulose diet group) as well as its role in the present results on arsenic metabolism.

CONCLUSION

The Hijiki diet accelerated and enhanced arsenic accumulation in rat blood cells, and faster decrease in other tissues than did a similar cellulose diet.

Acknowledgements The authors express their great appreciation to Mr Y. Nakano of the Research Reactor Institute, Kyoto University, for the activation analysis measurements, and to Professor A. A. Benson of The Scripps Institution of Oceanography, University of California, for discussions and review of the manuscript. Activation analysis was performed under the visiting research program at the Research Reactor Institute, Kyoto University.

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