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Arsenic distribution in rats fed a Hijiki diet

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Very large doses of sodium arsenate (Na_2HAsO_4), 14 mg As kg^{-1} of body weight, were administered to Sprague–Dawley male rats (body weight 300 g) fed a 5% Hijiki diet by stomach tube twice within two days. After 24 h, the rats were sacrificed and various organs were dried for subsequent neutron activation analysis. The distribution of arsenic (As) in selected organs was determined by neutron activation analysis. The highest concentration of As was found in blood cells with a rather high concentration in the liver and heart. As the control, rats which were fed on a 5% cellulose diet were used. Control rats which were administered arsenate showed that the arsenic distribution and the concentration in their organs were similar to those on the 5% Hijiki diet. Even the blood cells of the controls without any arsenic administration were found to contain a small amount of arsenic.

Keywords: Arsenic metabolism, rats, Hijiki diet, distribution of arsenic, blood cells, liver, heart, neutron activation analysis

INTRODUCTION

In a previous paper,[1] we reported analytical results on the administration of trace amounts of arsenate to rats. The rats retained about 70% of administered arsenic (As) in the body with a half-life of 120 days and 30% of administered arsenic with a half life of 0.4 days.

Whilst a smaller amount of arsenic is required by young animals for normal growth,[2] high arsenate levels are a hazard to animals.[2]

It has been reported that, of the seaweeds,[3,4] Hijiki contained a rather high level of arsenic.[3] The Japanese population consumes seaweeds including Hijiki at levels as high as 5.9 g day^{-1}.[5] The effect of some components in seaweed on arsenic distribution in the various organs is interesting from the viewpoint of biochemical and ecological nutrition, especially on the average consumption of Hijiki in Japan is 1 g day^{-1}.

In this study, rats were fed a diet containing Hijiki, and/or a very large dose of arsenate was administered. The distribution of arsenic (As) in various organs was determined by neutron activation analysis and it was found that arsenic accumulated in the blood cells in the highest concentration. The level of arsenic in the blood cells was significantly little different between rats fed a Hijiki diet and those fed a cellulose diet, after the administration of a very large dose of arsenate.

EXPERIMENTAL

Animals

Sprague–Dawley male rats, five weeks old, were divided into two groups. One group was fed a 5% Hijiki (Grade 1) diet [designated as group **H(1)**] for two weeks, and the other group was fed a 5% cellulose diet (designated as group **C**), the control. After two weeks, each group was further divided into two groups; one was administered arsenic [designated as group **H(1)-As** and group **C-As**] and the other was not [designated as group **H(1)-n** and group **C-n**]. Another separate group was fed 5% Hijiki (Grade 2) for two weeks [designated as group **H(2)-n**]. Each group included four or more rats. Each analysis was performed in triplicate. Hijiki Grade 2 contained more arsenic than Hijiki Grade 1; these grades are described below, under 'Arsenic concentration in the diet'.

Arsenic administration

Sodium arsenate, Na_2HAsO_4, was dissolved in water (0.7 mg As cm^{-3}). A dose of 14 mg As kg^{-1} body weight was administered (7 mg As kg^{-1}, once daily) by stomach tube for two days (group **H-As** and group **C-As**).

The rats were sacrificed 24 h after the last administration, and various organs were removed, dried and pulverized for arsenic analysis.

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Table 1 Arsenic concentration in various organs (ppm)[b]

Diet	Group	Blood cells	Liver	Heart	Lung	Kidney	Muscle	Bone	Testis
5% Hijiki (Grade 1) −As[a]	**H(1)-n**	5.47±0.77	0.71±0.82	1.62±1.02	0.67±0.77	0.36±0.27	0.16±0.19	0	1.13±0.88
5% Hijiki (Grade 1) +As	**H(1)-As**	26.48±2.71	13.45±3.17	20.26±6.21	27.05±2.24	14.14±3.03	0.69±0.26	0.56±0.23	0.40±0.19
5% Cellulose −As	**C-n**	1.30±0.40	0	0	0.6±0	0	0	0	0
5% Cellulose +As	**C-As**	24.65±0	7.87±1.63	10.91±1.89	18.57±4.06	9.96±2.07	1.32±0.64	0.44±0.20	0.91±0.61

[a] After being fed on 5% Hijiki (Grade 1) diet or 5% cellulose diet for two weeks, rats of each diet category were divided into two groups: one of these groups was administered 7 mg As kg^{-1} body weight (once daily) twice in two days (designated '+As'). The rats not admistered arsenic were designated as '−As'. After 24 h of the last arsenic administration, rats were sacrificed. Various organs were separated, dried and pulverized for neutron activation analysis.

[b] Mean ± SEM. More than four rats were used in each group. Each sample was analyzed in triplicate.

Thermal neutron activation analysis

The samples were irradiated in a flux of 10^{13} neutrons cm^{-2} s^{-1} in the nuclear reactor of the Research Reactor Institute, Kyoto University. Gamma radiation from ^{76}As was determined using a Ge/Li detector at 559.1 keV. Energy levels of ^{60}Co and ^{137}Cs was used for calibration; 1 μg of As gave about 10^3 counts.

Standardization of arsenic determination

Several quantitative amounts of arsenic were spotted on respective pieces of paper and irradiated together and separately with the samples in one polyethylene capsule. After a cooling time of one to three days, the radioactivity was determined by a γ-ray detector.

Diet composition

The composition of the diet was as follows: 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 lactate, 35.09%; iron citrate, 3.18%; $MgSO_4.3H_2O$, 7.17%; $ZnCO_3$, 0.11%; $MnSO_4$, 0.12%; $CuSO_4$, 0.03%; and KI, 0.01%.

The vitamin mixture 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, 520 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 collected on the shore of Goto islands, Kyusyu. The leaves of Hijiki were separated from the stems, and dried. Before use, the dried Hijiki was washed with water, dried at room temperature and pulverized.

RESULTS

Neutron activation analysis

Bromine-82 produced from ^{81}Br by neutron irradiation emits 554.3 keV γ-radiation. Thus, higher concentrations of bromine (Br) would interfere with measurement of ^{76}As at 559.1 keV. The amount of Br is required to be determined. However in our experiments there was no interference from Bromine-82.

Distribution of arsenic in blood cells (Table 1)

Arsenic was largely located in the red blood cells and undetectable in the serum. In the group **H(1)-As**, approximately 27 ppm of arsenic was

Table 2 Arsenic concentration in various organs (ppm)[b]

Diet	Group	Blood cells	Liver	Heart	Lung	Kidney	Muscle	Bone	Testis
5% Hijiki (Grade 2) −As[a]	**H(2)-n**	56.00 ± 24.10	3.00 ± 1.60	9.30 ± 3.80	10.20 ± 2.60	5.80 ± 1.70	0.20 ± 0.20	0	1.10 ± 0.90

[a] Hijiki (Grade 2) contained a higher concentration of arsenic, 70 μg As g^{-1} dry weight. The feeding conditions were as described in Table 1, without administration of arsenic.
[b] Mean ± SEM.

retained in the blood cells 24 h after administration. The group **C-As** had a similar level of arsenic in the blood cells. The group **H(1)-n** showed a 5.5 ppm level of arsenic, which could have come from the Hijiki containing a lower level of arsenic. The blood cells of the **C-n** group contained 1.3 ppm of arsenic even though the cellulose diet was As-free.

The rats fed on the Hijiki diet containing a higher level of arsenic (group **H(2)-n**) for two weeks accumulated a rather high level of arsenic in the blood cells. For these rats taking up about 70 μg of arsenic every day, it was calculated that they were fed about 900–1000 μg of arsenic in total. In these blood cells, 50 ppm of arsenic was detected (Table 2).

Distribution of arsenic in other organs (Tables 1 and 2)

Arsenic was accumulated in the liver, heart and lung as well, by groups **H(1)-As** and **C-As**. The perfused liver and heart also showed a higher level of arsenic. It is significant that the liver and heart have some affinity for arsenic, even though a small amount could have resulted from contamination with red blood cells. For the group **H(1)-n**, arsenic accumulated to a level of 1.6 ppm in the heart, and 0.7 ppm in the liver and lung (Table 1). For the group **C-n**, 0.6 ppm in lung and 1.3 ppm in spleen were detected in the various organs.

The **H(2)-n** group of rats accumulated arsenic in lung and liver tissues at levels similar to group **H(1)-As** and **C-As** (Table 2).

Percentage distribution of arsenic in the various organs

About 7% of the administered arsenic accumulated in the red blood cells in *Hijiki-* and cellulose-fed rats (groups **H(1)-As** and **C-As**). In other organs, less than a few per cent was detected (Table 3).

Arsenic concentration in the feces

In the feces of the groups **H(1)-As** and **C-As**, 13.15 ± 3.09 ppm (mean ± SEM) and 32.16 ± 7.54 ppm of arsenic was found, respectively. In total, 1.2% (group **H(1)-As**) and 3.5% (group **C-As**) of the administered arsenic was detected; this means that most of the administered arsenic was absorbed. The stomach and intestinal contents were less than 0.1% of the administered arsenic. In the feces of the group **H(2)-n**, 16.2 ± 2.2 ppm of arsenic was detected.

Arsenic concentration in the diet

The arsenic content of Hijiki (Grade 2) was 70 μg As g^{-1} dry weight. Hijiki (Grade 1) contained less than a few parts per million. On the other hand, arsenic was undetectable in the 5% cellulose diet.

DISCUSSION

A continuous supply of dietary arsenic seemed to result in accumulation in the red blood cells, as shown by the arsenic concentration in the blood

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

Diet		Group	Blood cells	Liver	Heart	Lung	Kidney
5% Hijiki (Grade 1)	+As	**H(1)-As**	6.47[c]	1.48	0.17	0.21	0.27
5% Cellulose	+As	**C-As**	6.79[c]	0.67	0.05	0.19	0.19

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

Table 4 Percentage distribution of arsenic in the feces and digestive tract contents (%)[a, b]

Diet		Group	Feces	Content		
				Stomach	Small intestine	Large intestine
5% Hijiki (Grade 1)	+As	**H(1)-As**	1.15	0.03	0.02	0.10
5% Cellulose	+As	**H(1)-As**	3.45	—	0.04	0.16

[a] The feeding conditions were as described in Table 1. The feces after arsenic administration were collected, dried and pulverized for the activation analysis. The digestive tract contents were stored when the organs were separated. [b] The percentage was expressed on the basis of the amount of arsenic administered.

cells of the H(1)-n and C-n groups of rats. The difference of arsenic concentration in the blood cells between the H(1)-n and the C-n groups suggests the different level of arsenic in the diets (Table 1), although the possibility of some components in Hijiki affecting arsenic accumulation has not been excluded.

As described in a previous paper,[1] 70% of administered arsenic (trace amount) was retained with a half life of 120 days. In the present experiment, where a very large dose of arsenic was administered, the arsenic distribution recovered in the various organs does not total 100% (Table 4). A large portion of the arsenic might be excreted to the urine, because of the limited capacity for retaining arsenic in the whole body. The remaining arsenical tissues must be examined as well. Otherwise, the remained arsenic might then spread into the whole body in lower concentration.

The red blood cells of rats have an extraordinary affinity for arsenic but with a limited capacity. According to tracer experiments, the arsenic released from protein by dithiothreitol was small.[1] The chemical form of the retained arsenic[6] remains to be elucidated, in comparison with the form of arsenic in marine diatoms.[7] Our preliminary experiments under the same conditions as those for rats indicate that the distribution of arsenic in the various organs of the mouse was different from that of the rat.

CONCLUSION

After the administration of arsenic, arsenic distribution in various organs of rats fed a Hijiki diet (containing a lower level of arsenic), showed mostly a similar pattern to those fed a cellulose diet. The highest arsenic concentration was found in the red blood cells, and rather lower concentrations were in the heart, lungs and liver. The rats fed Hijiki diets, containing higher levels of arsenic (70 ppm), accumulated about 50 ppm of arsenic within 2 weeks, mainly in the blood cells.

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