Trace Nutrients Research 36 : 54－57 (2019)

Original Article

Arsenic distribution in organs of rats fed brown algae, Akamoku (*Sargassum horneri*).

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(Received 2 September 2019; accepted 27 September 2019)

Summary

Sprague-Dawley male rats, 5 weeks old, were fed Akamoku diet or control diet for two weeks. Arsenic accumulation levels in several organs and the blood were determined by thermal neutron activation analysis. The highest concentration of arsenic was found in blood cells, followed by the spleen, lung, liver, kidney, and heart, in this order. Rats fed the control diet showed trace amounts of arsenic in the respective organs, with similar accumulation trends to those of the Akamoku diet group. These results are discussed with reference to findings from a previous study on radioactive inorganic arsenic in the whole body of the rat. The finding of arsenic in diet being transferred to various organs as well as to blood cells indicates the need to reduce the arsenic level in food as much as possible during processing procedures.

Keywords: Akamoku, arsenic, rat, thermal neutron activation analysis

Introduction

Consumption of the brown alga, Akamoku (*Sargassum horneri*), containing a high amount of dietary fiber, has been greatly increasing in Japan recently. Akamoku is a member of the Hijiki family, *Sargassum fusiforme,* which contains a rich amount of nutritionally beneficial minerals[1)], but also arsenic[1, 2)]. The effect of arsenic derived from Akamoku in animal bodies is not yet clear. In this study, we investigated the effect of arsenic distribution in various organs of rats fed Akamoku.

Methods

1. Plant samples

Akamoku, *Sargassum horneri,* plants, were harvested on the sea coast of Ohshima Matsugashita, Fukuoka, Japan. Plants growing in sea water at a depth of 5 m were harvested.

2. Sample preparation

The harvested fresh Akamoku plants were washed 3 times with distilled water, blotted, lyophilized and pulverized. The fresh samples of Akamoku contained 89.1% (average) of water before lyophilization. The dried samples were pulverized with a mill. The dried Akamoku, rich in dietary fiber, consisted of 57% dry matter[3)].

Table 1 Diet compositions (%)

Component	Akamoku diet	Control diet
Corn starch	46.57	46.57
α-Corn starch	15.5	15.5
Casein	14.0	14.0
Sucrose	10.0	10.0
Soybean oil	4.0	4.0
Cellulose	**0.0**	**5.0**
Akamoku	**5.0**	**0.0**
Mineral mixture	3.5	3.5
Vitamin mixture	1.0	1.0
L-Cystine	0.18	0.18
Choline hydrogen tartrate	0.25	0.25
t-Butylhydroquinone	0.0008	0.0008

The mineral mixture and vitamin mixture were prepared according to the AIN-M formulation. The pulverized Akamoku was prepared as described in the text.

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3. Diet compositions

The diet was prepared according to the AIN-M formulation. The control diet contained 5% cellulose and in the Akamoku diet, included 5% of pulverized dried Akamoku (Table 1).

4. Animals

Sprague Dawley male rats, 4 weeks old, were fed the solid diet for one week and then fed either (1) the diet containing pulverized Akamoku plants or (2) the standard diet (AIN-M) for 2 weeks. Water and diet were available *ad libitum*. The animal experiment in the present study was approved by the Ethical Committee of Fukuoka Women's University.

5. Determination of arsenic in the samples

The samples of the respective organs were lyophilized, and the dried specimens were sealed respectively in polyethylene bags, bundled and put in polyethylene Neuma-capsules.

6. Thermal neutron activation analysis[4, 5]

The specimens, sealed in polyethylene Neuma-capsules were irradiated in a flux of 10^{13} neutrons · cm^{-2} · sec^{-1} for 20 min in the center position of the nuclear reactor of the Research Reactor Institute, Kyoto University. After cooling for 72 hr, the arsenic contents in the samples were determined by gamma radiation from ^{76}As using a pure Ge gamma-detector at 559.1 keV. The energy levels of ^{60}Co and ^{137}Cs were used for the calibration.

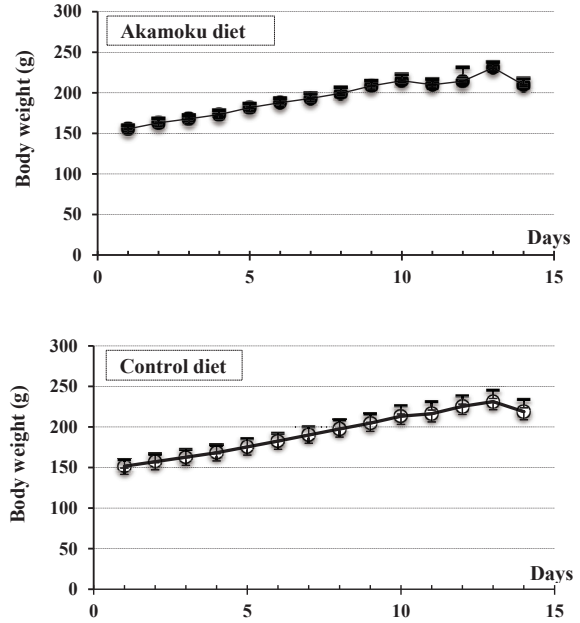

Fig. 1 Growth curves of rats fed Akamoku diet and control diet. Bars express the standard deviation.
On the 14th day, the body weight decreased due to fasting on the last day.

7. Statistics

The statistical values were expressed as average values ± standard deviations, and arsenic values were determined by duplicate or triplicate measurements. For comparison of the values, the t-test was used.

Results and discussion

1. Animal growth curves

The growth curves of rats were not significantly different between the control and Akamoku diet groups (Fig. 1). No effect of Akamoku components was found during the 2 weeks, indicating that Akamoku does not contain any special components affecting physiological levels. The body weight of the Akamoku group on the first day, 155.4 g, increased to 230.7 g on the 13th day and decreased to 209.5 g on the 14th day due to fasting; the values of the control group changed from 151.6 g to 231.3 g and 218.9 g on the 14th day after fasting. As the growth curves of the Akamoku diet group and that of the control were not different, the arsenic in the diet did not seem to affect the body weight gains; that is, metabolism related to body weight gain was not affected during this period.

Daily food intake was 18.3 ± 1.3 g/day, without a significant difference from the control (19.3 ± 0.3 g/day). The water intake did not differ between the two groups.

2. Arsenic contents in diets

The arsenic amounts in the diets, shown in Table 2, were taken daily and accumulated in the respective organs.

3. Organ weights

The weights of the organs of the two groups were not modified by Akamoku components (Table 3), in spite of the markedly higher amount of arsenic that accumulated in some organs. Thus, the arsenic level in the organs did not appear to affect the general metabolism in the respective organs during the experimental period.

4. Arsenic accumulation in the respective organs (Table 4)

The arsenic concentrations that accumulated in the **spleen** were 71.4 ppm on the dry weight basis in the Akamoku group and 1.6 ppm on the dry weight basis in the control diet group. The arsenic concentration that accumulated in the **lung** was 44.5 ppm and that in the **liver** and **kidney** was 20.4 ppm and 21.4 ppm respectively in the Akamoku diet group. The arsenic concentration in the **heart** was 13.7 ppm and in the **femur** was 5.6 ppm in the

Table 2 Intake of diet and arsenic per day

Diet	Diet (dry weight, g/day)	As (μg/day)
Akamoku diet	18.28 ± 1.28	163.65 ± 11.42
Control diet	19.28 ± 0.25	1.81 ± 0.03

Table 3 Weights of organs examined (g of dry weight)

Diet \ Organ	Liver	Kidney	Spleen	Heart	Lung	Femur
Akamoku diet	1.23 ± 0.16	0.37 ± 0.09	0.13 ± 0.02	0.16 ± 0.01	0.22 ± 0.03	0.13 ± 0.02
Control diet	1.04 ± 0.36	0.40 ± 0.04	0.12 ± 0.05	0.17 ± 0.01	0.21 ± 0.03	0.14 ± 0.02

Table 4 Arsenic contents in organs examined (μg As/g dry weight of tissues)

Diet \ Organ	Liver	Kidney	Spleen	Heart	Lung	Femur
Akamoku diet	20.42 ± 6.39	21.44 ± 5.78	71.35 ± 20.70	13.68 ± 1.94	44.45 ± 5.48	5.59 ± 0.78
Control diet	0.48 ± 0.07	0.54 ± 0.28	1.56 ± 0.31	0.33 ± 0.00	1.15 ± 0.00	0.42 ± 0.07

Table 5 Arsenic accumulation in blood cells and serum

Diet	Blood cells: As content (μg As/g dry weight)	Serum: As content (μg As/ml)
Akamoku diet	501.28 ± 27.13	9.71 ± 7.20
Control diet	12.76 ± 0.81	2.14 ± 0.00

Table 6 Arsenic concentrations in feces and urine

Excreta / Diet	Feces: As content (μg As/g dry weight of feces)	Urine: As content (μg As/ml of urine)
Akamoku diet	32.58 ± 5.55	16.41 ± 8.95
Control diet	0.11 ± 0.03	0.09 ± 0.00

Akamoku group. In the control diet group, these organs had accumulated less than a few ppm of arsenic.

As shown in Table 5, the arsenic concentrations that had accumulated in blood cells were 501.3 ppm in the Akamoku diet group and 12.8 ppm in the control diet group. The arsenic concentrations in **serum** were 9.7 ppm in the Akamoku diet group and 2.1 ppm in the control diet group. The arsenic levels in the **blood cells** and the **spleen** coincide with the mechanism that rats possess of detoxicating arsenic by binding it with the cysteine[93] of hemoglobin, a site that is not related to oxygen transport by the blood[6].

5. Arsenic concentrations in feces and urine (Table 6)

The arsenic concentrations excreted in **feces** were 32.6 μg As/g dry weight of feces for the Akamoku diet group and 0.1 μg As/g dry weight of feces for the control diet group.

The arsenic concentrations excreted via the **urine** were 16.4 μg As/ml of urine for the Akamoku diet group and 0.1 μg As/ml of urine for the control diet group.

6. Accumulation of arsenic in the rat body

Of the components in the Akamoku plants, arsenic displays the most remarkable behavior; even a trace amount of arsenic in the control diet was transferred to the organs with a similar distribution ratio among the respective organs. The total amount of arsenic that accumulated in the rat body was about 70% of the total amount of the Akamoku diet and 44% of the control diet.

The behavior of arsenic has been observed by administering radioactive inorganic arsenic (^{74}As) into the vein of rats[7]; 30% of the total of the administered arsenic was retained in the body with a half life of 0.4 day, and 70% of the total arsenic was retained in the whole body with a half life of 100 to 120 days.

Conclusion

As arsenic from Akamoku plants accumulated in the body, we need to find a simple and effective way to reduce the arsenic content in Akamoku plants in the pre-cooking process, without large loss of the nutritionally beneficial mineral elements.

Acknowledgements

The authors express their sincere acknowledgement to the late Mr. Yukihiro Nakano of Research Reactor

Institute, Kyoto University for his technical support in the arsenic determination by neutron activation analysis. The authors also express their appreciations to Ms. Yayoi Kamogawa of Fukuoka Women's University for her assistance in feeding the rats, and to Ms. Kana Ogawa and Kumiko Kamiya of Gifu Women's University for their assistance in the preparation of Akamoku samples for analysis.

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