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Acute Effects of Orally Administered Sodium Arsenate on Heme Biosynthetic Enzymes in the Tissues of Three Strains of Mice†

Masao Kondo[1]* and Isamu Ichikawa[2]
[1]Department of Nutrition and Biochemistry, National Institute of Public Health, Tokyo 108-8638, Japan
[2]Department of Industry Health[b], National Institute of Public Health, Tokyo 108-8638, Japan

Sodium arsenate ($Na_2HASO_4 \cdot 7H_2O$, As(V)) was administered to three genetically different strains of mice, ICR, C57BL/6J and DBA/2J, in a single oral dose of 54 mg kg^{-1} body weight. The effects on the activities of heme biosynthetic enzymes in the spleen, liver, kidney and peripheral blood were investigated. The activities of δ-aminolevulinate synthase (ALAS, EC 2.3.1.37), catalyzing the first reaction in the heme biosynthetic pathway and the rate-limiting enzyme for heme synthesis, δ-aminolevuilinate dehydratase (ALAD, EC 4.2.1.24) and porphobilinogen deaminase (PBGD, EC 4.3.1.8) in the spleens of the ICR strain, were remarkably reduced to 51%, 32% and 42%, respectively, in comparison with each mean value of the control group. In contrast, these activities were significantly increased in the livers of the C57BL/6J strain, and a similar trend was observed in the DBA/2J strain. In the kidneys, the ALAD activity was significantly reduced in the ICR strain, but no significant differences in the other two strains were found.

These results suggest that the influence of As(V) on the activity of heme biosynthetic enzymes is most potently manifested in the spleen tissue of the ICR strain.

Keywords: arsenic compound; heme biosynthesis; δ-aminolevulinate synthase; mouse strain; spleen

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* Correspondence to: Masao Kondo, National Institute of Public Health, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8638, Japan.
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INTRODUCTION

It is well known that metals inhibit the activities of the heme biosynthetic enzyme system. In particular, δ-aminolevulinate dehydratase (ALAD) activity in the second enzyme in the pathway has been shown empirically, in both *in vitro* and *in vivo* studies, to be inhibited by many metals.[1] Since the ALAD activity of peripheral blood is strongly inhibited by lead, this activity is used widely as an index of exposure to its low concentrations.[1]

Recently, there have been several occurrences of inorganic arsenic contamination of drinking water in several countries, including India, China and Mexico,[2,3] resulting in an increase in the incidence of chronic arsenic poisoning and leading to major social problems. In contrast, there have been few reports of studies on the influence of inorganic arsenic compounds (As) on the porphyrin metabolic enzymes. It has been reported that urinary porphyrin levels in animals and humans increased after exposure to As.[3–7] These findings point to the adverse effect of As on heme biosynthetic enzyme activities and suggest the potential of this system as a biomarker for the detection of early-stage As toxicity.[8] In our previous investigations on Wistar rats and ICR mice, we found that the effects of a single oral administration of As(V) on heme biosynthetic enzyme activities varied in magnitude from one animal species to another and between the sexes and organs or tissues within a given species.[9–11] In particular, we found that administration of As(V) markedly decreased the activities of the heme biosynthetic enzymes in the spleen of mice of the ICR strain,[9,11] although little effect on these activities was noted in rat bone marrow cells.[10]

It is well known that among several strains of mice, the selection of a suitable strain for use in a particular experiment depends upon the objective of the study.[12–15] Gross and Hulton[14] measured the activity of δ-aminolevulinate synthase (ALAS) in

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15 strains of inbred mice and reported finding little difference among them. In contrast, they found a strain-specific variation in this activity using experimental 3,5-dicarbethoxy-1,4-dihydro-2,4,6-trimethylpyridine (DDC)-induced experimental porphyria. Furthermore, in an investigation of the ALAD activity in 10 differing strains of mice, Doyle and Shimke[13] reported finding an approximately eight-fold higher activity in the spleens and an approximately three-fold higher level in the livers and kidneys of the DBA/2J strain than the C57BL/6J one. From these findings, it could be surmised that the activity levels of the porphyrin-metabolizing enzymes vary in magnitude from one strain of mouse to another, and that concomitant differences will exist in the effects of chemical substances on these activities.

Accordingly, in the present study, we investigated the influence of an As(V) compound on the activity of three heme biosynthetic enzymes, ALAS, ALAD and Porphobilinogen deaminase (PBGD), by using three genetically differing strains of male mice.

MATERIALS AND METHODS

Chemicals

Sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) was obtained from the Wako Pure Chemicals Company (Tokyo). ALA was obtained from the Daiichi Pure Chemicals Company (Tokyo). Porphobilinogen (PBG), protoporphyrin (PROTO) and zinc protoporphyrin (Zn-PROTO) were obtained from Porphyrin Products (Logan, UT, USA). Other chemicals used were of the purest available reagent grade.

Animals and treatment

Animals used were four-week-old male mice from the Jcl:ICR, C57BL/6J Jcl and DBA/2J Jcl strains (Shizuoka Laboratory Animals Co. Ltd, Hamamatsu, Japan). These animals were allocated to six groups, each containing five or six animals, and received a single oral dose of sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$, As(V)), at 54 mg ml^{-1} kg^{-1} body weight. The injection volume was used in order to average the body weight of the mice in each group. Each control mouse received the same volume of 5% glucose solution without the metals. All animals were housed in stainless steel cages in an air-conditioned room at 24 ± 1 °C, with free access to a normal commercial diet and water for 18 h.

Enzyme preparation from liver, kidneys, spleen and blood

Mice were sacrificed under ether anesthesia.[10,11] Heparinized blood was collected and the liver perfused through the portal vein with cold saline. Kidneys and spleens were carefully removed and weighed. Liver, kidneys and spleens were homogenized in 0.25 M sucrose solution (containing 0.01 M potassium phosphate, 0.01 M $NaHCO_3$, pH 8.0). Supernatant and precipitation fractions were prepared from the homogenates by centrifugation at

Table 1 Effects of sodium arsenate (Na_2HAsO_4) on the weights of body, liver, spleen and kidney of different strains of male mice

	Body weight (g)		Liver weight (g)	Spleen weight (g)	Kidney weight (g)
	Before	After			
ICR Jcl					
Control	29.8 ± 2.03	31.3 ± 0.95	2.80 ± 0.13	0.13 ± 0.03	0.51 ± 0.02
$Na_2HAsO_4 \cdot 7H_2O$	27.9 ± 0.49	29.8 ± 0.98*	2.00 ± 0.22**	0.09 ± 0.01*	0.49 ± 0.04
C57BL/6J Jcl					
Control	18.7 ± 0.54	19.8 ± 0.22	1.50 ± 0.11	0.06 ± 0.01	0.22 ± 0.01
$Na_2HAsO_4 \cdot 7H_2O$	18.9 ± 0.80	19.7 ± 0.60	1.30 ± 0.15	0.05 ± 0.00	0.25 ± 0.02**
DBA/2J Jcl					
Control	16.6 ± 0.36	17.3 ± 0.57	0.90 ± 0.12	0.06 ± 0.01	0.22 ± 0.01
$Na_2HAsO_4 \cdot 7H_2O$	16.8 ± 0.75	16.6 ± 0.72	0.90 ± 0.12	0.05 ± 0.01	0.23 ± 0.02

Values represent means ± SD of five or six mice per group. Significant differences from corresponding controls (Student *t*-test):
* $P < 0.05$;
** $P < 0.01$.

Table 2 Contents of hemoglobin, hematocrit and porphyrins in peripheral blood

	Hemoglobin (g/dl)	Hematocrit (%)	Porphyrins (μg/dlRBC)	
			Zn-PROTO[a]	Free PROTO
ICR Jcl				
Control	13.9 ± 0.52	41.5 ± 1.54	104.9 ± 15.3	28.8 ± 9.89
$Na_2HAsO_4 \cdot 7H_2O$	13.5 ± 0.10	40.4 ± 2.75	88.4 ± 14.2	17.6 ± 4.80
C57BL/6J Jcl				
Control	13.0 ± 0.65	43.5 ± 1.48	60.4 ± 11.4	25.8 ± 4.44
$Na_2HAsO_4 \cdot 7H_2O$	12.9 ± 0.84	43.3 ± 0.76	69.2 ± 12.9	32.2 ± 5.06
DBA/2J Jcl				
Control	13.8 ± 0.49	42.6 ± 1.75	83.7 ± 5.9	37.8 ± 6.95
$Na_2HAsO_4 \cdot 7H_2O$	13.5 ± 0.23	41.4 ± 1.45	81.3 ± 17.0	28.8 ± 9.86

Values represent means $\pm$ SD of five or six mice per group.
[a] PROTO, protoporphyrin.

48 800*g* for 30 min.[10,11] The precipitation fraction was further washed twice with 0.25 M sucrose solution, resuspended in the same solution and used for the ALAS assay. The supernatant fraction and whole-blood lysates were used for the assay of ALAD and PBGD.

Enzyme assay

The activities of ALAS,[16] ALAD[17] and PBGD[18] were assayed according to the methods described previously.

Other procedures

Protein was determined by the method of Lowry *et al*.[18] with a modification.[19] Erythrocyte porphyrins were determined by the method reported previously.[20] The hematocrit value was determined by a capillary tube method.

The means and the SD were calculated and the statistical significance of the differences between the treated and the control groups was determined using Student's *t*-test.

RESULTS

Weights of body, liver, spleen and kidneys

In comparison with the control group, weights of body, liver and spleen of ICR mice decreased significantly after the administration of As(V). However, the kidney weight of C57BL/6J mice increased significantly relative to the control group (Table 1).

Contents of hemoglobin (Hb), hematocrit (Ht) and porphyrin values of peripheral blood

No influence of As(V) administration was observed on Hb content and Ht values in any strain of mice, but a tendency to decrease was seen in the porphyrin content of the erythrocytes of ICR mice (Table 2).

ALAS activity in spleen and liver

The ALAS activity in the spleens of the control ICR mice was 0.77 ± 0.23 nmol ALA formed (mg of protein)$^{-1}$ h^{-1} which was approximately five times higher than that (0.16 ± 0.04 nmol ALA formed (mg of protein)$^{-1}$ h^{-1}) in the spleens of the C57BL/6J mice. The ALAS activity in the spleens of the ICR mice after the As(V) administration was 0.39 ± 0.22 nmol ALA formed (mg of protein)$^{-1}$ h^{-1} representing a decrease of approximately 49%. Significant differences in the ALAS activity in spleens in the C57BL/6J and DBA/2J mice were not observed. Only negligible differences among the three strains of mice used were observed in the ALAS activity in the livers of the controls, but after the As(V) administration, significant elevations ($P < 0.01$) in the hepatic ALAS activity in the C57BL/6J and DBA/2J mice were observed at approximately 1.7 times and 1.4 times the control level, respectively (Fig. 1).

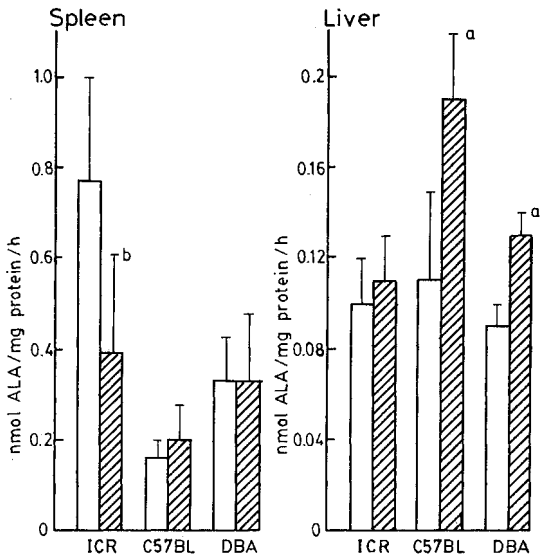

Figure 1 Effect of sodium arsenate on δ-aminolevulinate synthase activity in spleen and liver of different strains of male mice: □, control group; ▨, As(V)-treated group. Data presented are the means ± SD from five or six mice per group. Significant difference from control: a, $P < 0.01$; b, $P < 0.05$.

ALAD activity in peripheral blood, spleen, liver and kidneys

The ALAD activity level in the peripheral blood, spleen, liver and kidneys of each control group of the DBA/2J, ICR and C57BL/6J mice was high (Fig. 2). The inhibitory effect of As(V) administration on the activity in the peripheral blood and the spleen showed a similar pattern, but the effect was much more pronounced in the spleen than the erythrocytes. Levels of the activity in the As(V)-treated ICR and DBA/2J mice were reduced to 68% and 31% of each control value, respectively. Hepatic ALAD activity was significantly elevated in the C57BL/6J mice of the As(V)-treated group. Renal ALAD activity in the As(V)-treated DBA/2J mice showed the highest value, being approximately 6.7-fold higher than that in the treated ICR mice.

PBGD activity in peripheral blood, spleen, liver and kidneys

The PBGD activities in the peripheral blood, spleen, liver and kidneys are shown in Fig. 3. ICR mice had the highest values of PBGD activities in peripheral blood and spleen, but the lowest value in the kidneys. The effect of As(V) administration on the PBGD activity was similar to the effect on the ALAD activity, with a common inhibitory pattern in peripheral blood and spleens, but with a more pronounced inhibition in a spleens. Levels of activity in the As(V)-treated ICR and DBA/2J mice were reduced to 58% and 37% of each control

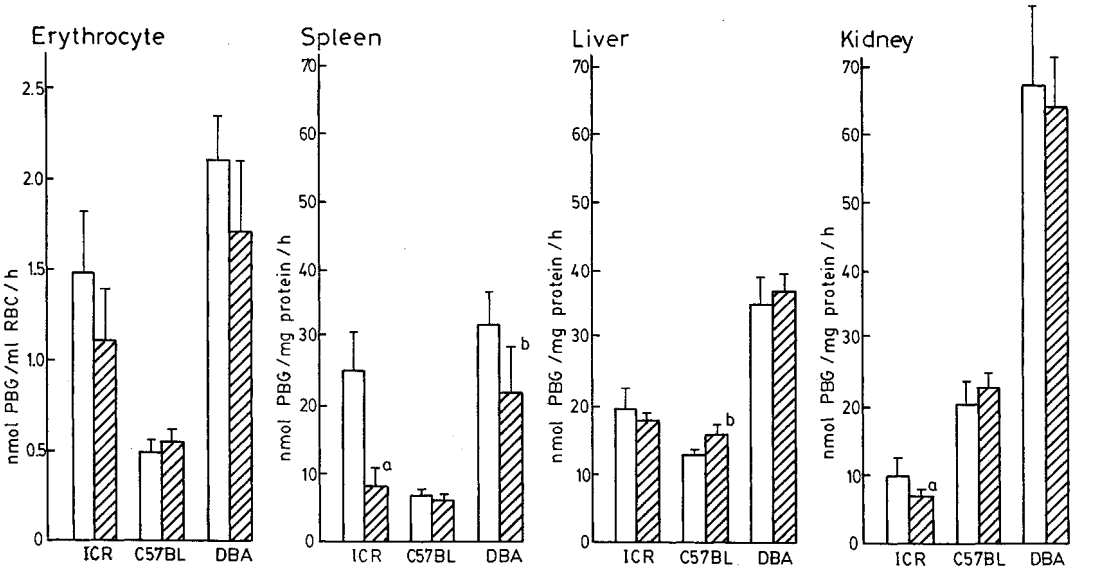

Figure 2 Effect of sodium arsenate on δ-aminolevulinate dehydratase activity in erythrocytes, spleen, liver and kidney of different strains of male mice: □, control group; ▨, As(V)-treated group. Data presented are the means ± SD from five or six mice per group. Significant difference from control: a, $P < 0.01$; b, $P < 0.05$.

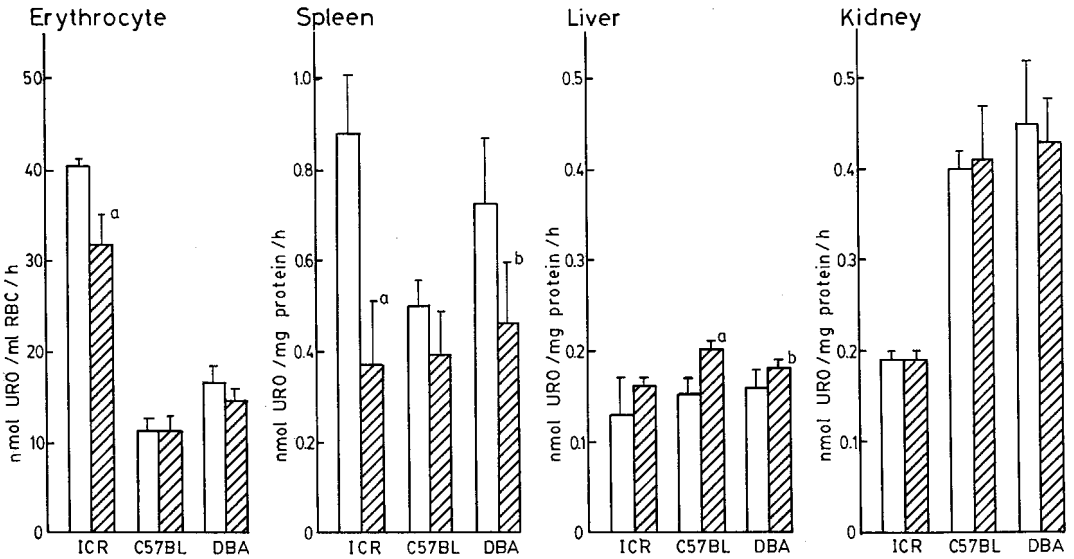

Figure 3 Effect of sodium arsenate on porphobilinogen deaminase activity in erythrocytes, spleen, liver and kidneys of different strains of male mice: □, control group; ▨, As(V)-treated group. Data presented are the means ± SD from five or six mice per group. Significant difference from control: a, $P < 0.01$; b, $P < 0.05$.

value, respectively. Moreover, the level of the PBGD activity in the liver was significantly elevated in all As(V)-treated mice of the C57BL/6J ($P < 0.01$) and DBA/2J ($P < 0.05$) strains. Levels of ALAD activity in the kidneys of the C57BL/6J and the DBA/2J mice were about two-fold higher than that of ICR mice, but no significant differences were observed after the administration of As(V).

DISCUSSION

It is well known that the administration of sodium arsenate [As(V)] to rats and mice leads to a remarkable increase in their urinary uroporphyrin levels.[4–7] Woods and Fowler.[4,5] reported observing a 12-fold increase in the urinary uroporphyrin of male Sprague–Dawley (SD) rats (CD strain) relative to control levels after allowing them to drink distilled water containing As(V) at a concentration of 85 ppm for six weeks. It can be surmised that this phenomenon probably derives from a decrease in hepatic uroporphyrinogen decarboxylase (UROD, EC4.1.1.37) activity (86% of the control level of activity), but there have been reports that hepatic ALAS activity is at a normal or decreased level. However, there has been very little differentiation in this effect between As(V) and As(III) (sodium arsenite).

Recently, it has been found that ALAS consists of two isozymes: non-specific ALAS (ALAS-N) and erythroid-specific ALAS (ALAS-E).[1,22] It has also become clear that ALAS-N is regulated via negative feedback by heme. Accordingly, in hexachlorobenzene-poisoned animals and porphyria cutanea tarda, with markedly reduced hepatic UROD activity, hepatic ALAS activity is elevated via a derepression mechanism deriving from a reduction in heme. This leads to an overproduction of the UROD substrate, uroporphyrinogen, which is excreted in large amounts in the urine.[1] Consequently, an increase in urinary uroporphyrin following the administration of As(V) can only be explained in terms of an elevation of hepatic ALAS activity.

Our previous animal studies on porphyrin metabolism after intratracheal or oral administration of As(V) using rats and mice have shown that the effects may vary with animal species,[10] sex[11] and tissue or organ type.[9–11] In particular, different levels of the activity of ALAS, ALAD and PBGD in hematopoietic tissues of rats and mice suggest that the regulatory mechanism of heme biosynthesis might not be the same among these animals.[9–11]

In this study, we discovered that levels of ALAS, ALAD and PBGD activity in the spleen were remarkably decreased after the administration of As(V) to ICR mice.[11]

It has been demonstrated that several differences

in the level of porphyrin-metabolizing enzymes exist among strains of mice.[12–15] In the present study, investigating influences of acute oral administration of As^{5+} on the activity of porphyrin-metabolizing enzymes in the representative mouse strains (ICR, C57BL/6J and DBA/2J), we found that the liver ALAS activity was significantly elevated in the C57BL/6J and DBA/2J mice (Fig. 1) and that the ALAD and PBGD activities were also elevated (Fig. 2). Although we did not measure the liver UROD activity in the present study, it is not unreasonable to predict that a reduction in its activity would be related to an increase in urinary uroporphyrin after As(V) administration, indicating that the C57BL/6J strain is an appropriate animal model for abnormalities in liver porphyrin metabolism arising from exposure to As(V). In contrast, the levels of ALAS, ALAD and PBGD activities in the spleens of the ICR mice showed a remarkable decrease. Although the regulatory mechanism of heme biosynthesis in hematopoietic cells remains speculative, and sideroblastic anemia is the only known disease in humans to be associated with a decrease in ALAS activity, this activity may serve as a useful animal model of this disease.[1] To the best of our knowledge, ours may be the first reported experiment using ICR mice that points to the potential usefulness of this strain as an animal model, which may shed further light on the underlying causative mechanism of hematopoietic dysfunctions.

These results indicate that the influence of As(V) administration on porphyrin metabolism varies among different strains of mice and tissues or organs, although the findings obtained to date clearly indicate that the effect of arsenic compounds in causing porphyrin metabolism abnormalities was stronger in hematopoietic cells than in hepatocytes. Future studies will need to investigate other heme biosynthetic enzymes such as UROD and ferrochelatase, in addition to ALAS, ALAD and PBGD.

Furthermore, arsenic compounds did not inhibit ALAS, ALAD and PBGD to any noticeable extent *in vitro* (data not shown). This points strongly to the possibility that the reduction in the activity of hematopoietic enzymes of the spleen after the administration of As(V) derives from a reduction in the amount of mRNA encoding these enzymes.

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