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Original Article

Species difference in antimony and arsenic metabolism between hamster and rat after administration of tri- or pentavalent inorganic antimony

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ABSTRACT — Antimony (Sb) is a useful metalloid in many industries and a therapeutic agent for leishmaniasis in developing countries. Thus, it is expected that humans and wild animals face the risk of exposure to Sb. Although Sb is generally recognized as a toxic element, the mechanisms underlying its toxicity have not been fully elucidated yet. The objective of this study was to evaluate species differences in Sb distribution in blood and urine between rat and hamster. Antimony was more preferably accumulated in rat red blood cells (RBCs) than hamster RBCs. On the other hand, it has been reported that arsenic is bound to a specific cysteine residue in rat hemoglobin, which results in the substantial accumulation of arsenic in rat RBCs. These have led us to formulate the hypothesis that Sb, which belongs to the same group in the periodic table as arsenic, is also accumulated in the same manner as arsenic. However, because Sb was less accumulated than arsenic even in rat RBCs, Sb seemed to have less affinity for the cysteine residue than arsenic. Trivalent Sb showed greater accumulation than pentavalent Sb in rat RBCs. Consequently, species differences in Sb distribution between rat and hamster could be attributed to the affinity for the specific cysteine residue in hemoglobin.

Key words: Antimony, Arsenic, Rat, Hamster, ICP-MS

INTRODUCTION

Antimony (Sb) belongs to group 15 in the period table, which includes such elements as nitrogen, phosphorus, and arsenic as well. Antimony has unique physicochemical properties because it exhibits the characteristics of a metal and a typical element, and thus, is widely used in industry as a flame retardant, a semiconductor dopant, and an anti-ablation agent in brake systems of heavy vehicles. In addition to its industrial uses, Sb is also used as a remedy for leishmaniasis, a parasitic disease mainly reported in developing countries (Guerin *et al*., 2002). The most common treatment for leishmaniasis has been based on pentavalent antimonial compounds, such as meglumine antimonate and sodium stibogluconate, which are named Glucantime and Pentostam in the market, respectively (Dorlo *et al*., 2012). Therefore, it is expected that human and wild animals face to risk Sb exposure. Although Sb is generally recognized as a toxic element, the mechanisms underlying its toxicity have not been fully elucidated yet. Antimony is speculated to have the same toxicity mechanism as arsenic, which belongs to the same group in the periodic table as Sb. Contrary to Sb, however, the toxic mechanisms of arsenic have been widely studied. One of the most crucial and interesting characteristics of arsenic toxicity is species difference. It has been reported that among rodents, the rat shows high tolerance to arsenic (Naranmandura *et al*., 2007). Due to the similari-

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ty in physicochemical properties between Sb and arsenic, it is speculated that the metabolism of these two elements would be analogous. On the other hand, both elements have their own biological and toxicological effects. For instance, arsenic trioxide is used for the chemotherapy of acute promyelocytic leukemia, whereas inorganic Sb compounds are not used for this purpose (Specchia *et al*., 2013). Therefore, the specific toxicity of Sb should be clarified.

In this study, we evaluated species differences in Sb distribution in blood and urine between rat and hamster because Sb distribution is an important signature of species difference. Although arsenic is not recognized as an essential element in animals, a substantial amount of arsenic is detected in animal blood and urine. Thus, changes in arsenic concentration in blood and urine were also evaluated.

MATERIALS AND METHODS

Chemicals

Potassium antimony tartrate (Sb(III)), potassium hexahydroxoantimonate (V) (Sb(V)), and nitric acid for metal analysis were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Saline was purchased from Otsuka Pharmaceutical Factory (Tokushima, Japan). Antimony (Sb) and arsenic standard solutions for elemental determination were purchased from Nacalai Tesque (Kyoto, Japan) and SPEX CertiPrep (Metuchen, NJ, USA), respectively.

Animals

All animal experiments were approved by the Animal Investigation Committee of Chiba University (28-60, 28/01/2016).

Specific pathogen free (SPF) male Wistar rats and Syrian hamsters (5 weeks of age) were purchased from Japan SLC (Shizuoka, Japan) and housed in a humidity-controlled room maintained at 25 ± 2ºC with a 12 hr light-dark cycle. The rats and the hamsters were fed a commercial diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum*. Four animals were allocated to each group. After a one-week acclimation period, the tap water was changed to Milli-Q water (18.3 MΩ cm) until animal sacrifice. Four rats or hamsters in each group (n = 4) were orally administered tri- or pentavalent antimony compounds at the dose of 5 mg Sb/kg body weight dissolved in saline, and the animals were euthanized 24 hr after administration. A control group was administered the same volume of saline (10 mL/kg). The animals were housed in metabolic cages to collect urine 24 hr before and after administration. The urine samples were stored at −20ºC prior to use.

Blood and urine sample preparation

Blood was collected from the abdominal aorta under anesthesia into a heparinized tube (Becton, Dickinson and Company; Franklin Lakes, NJ, USA). Hematocrit value was measured by centrifugation of whole blood at 1,600 × g for 10 min and stored in a hematocrit capillary tube (Hirschmann; Eberstadt, Germany). Remaining heparinized whole blood was collected and centrifuged at 1,600 × g for 10 min to obtain plasma. The plasma samples were stored at −20ºC prior to use.

A 500 μL aliquot each of the urine and whole blood samples, and a 100 μL aliquot of plasma sample were mixed with 500 μL of concentrated nitric acid in a glass test tube, and the mixture was ashed at 200ºC for one day until the solution became colorless. The ashed samples were diluted with Milli-Q water to the appropriate concentrations of the target elements.

Determination of elements by ICP-MS

The concentrations of Sb and arsenic in the samples were determined by ICP-MS (Agilent 8800, Agilent Technologies, Hachiouji, Japan) with a standard calibration method. Signal intensities of Sb and arsenic were monitored at m/z 121 and 75, respectively,

Statistical analysis

All determinations were performed in four replicates and the results are shown as means ± S.D. Statistical analysis was conducted by applying the two-way analysis of variance (ANOVA) with Bonferroni's multiple comparisons test.

RESULTS AND DISCUSSION

Antimony concentrations in blood and urine

Antimony concentrations in whole blood of rat treated with Sb were significantly higher than those of hamster (Fig. 1a). Although Sb concentrations in the plasma of rat treated with Sb were much lower than those of hamster, Sb concentrations in RBCs of rat were significantly higher than those of hamster (Figs. 1b and 1c). These results indicate that rat RBCs more preferably accumulate inorganic Sb than hamster RBCs. The species difference in Sb accumulation in RBCs between rat and hamster coincides with that observed for arsenic (Naranmandura *et al*., 2007). Rat hemoglobin α chain and β chain have a unique amino acid sequence, namely, there is a specific cysteine residue on its sequence that binds arsenic

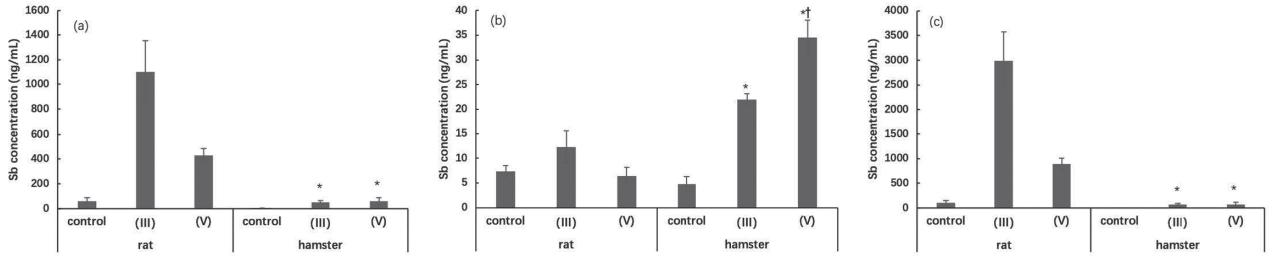

Fig. 1. Antimony concentrations in whole blood (a), plasma (b), and red blood cells (c) of Wistar rat and Syrian hamster orally administered potassium antimony tartrate (Sb(III)) and potassium hexahydroxoantimonate (Sb(V)). Data are expressed as means ± S.D. of 4 samples. * indicates significant difference between rat and hamster groups at $p < 0.05$. † indicates significant difference between Sb(III)- and Sb(V)-treated groups at $p < 0.05$.

(Lu *et al*., 2004). Antimony seems to be also bound to the unique cysteine residue in rat hemoglobin, resulting in the hyperaccumulation of Sb in rat RBCs. In addition, Sb(III) was more accumulated in rat RBCs than Sb(V) (Fig. 1c). Because Sb(III) has higher affinity for cysteine than Sb(V), this observation agrees with the role of hemoglobin in the species difference. It is still unclear whether there are specific proteins that bind Sb in hamster plasma or not. Speciation analyses are needed to explain why Sb concentrations in the plasma of hamster are higher than those of rat.

There were no significant differences in Sb concentrations in urine between rat and hamster, and between Sb(III) and Sb(V) (Fig. 2).

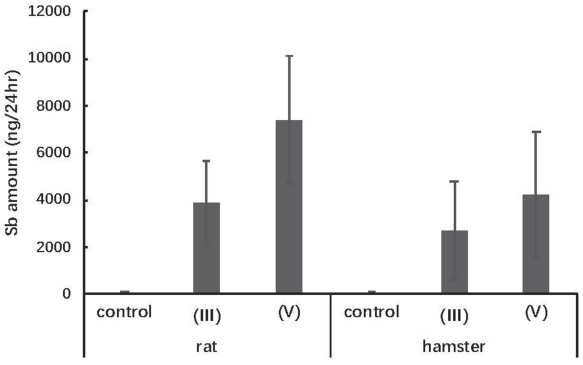

Fig. 2. Antimony levels in urine of Wistar rat and Syrian hamster orally administered potassium antimony tartrate (Sb(III)) and potassium hexahydroxoantimonate (Sb(V)). Data are expressed as means ± S.D. of 4 samples. * indicates a significant difference between rat and hamster groups at $p < 0.05$. † indicates a significant difference between Sb(III)- and Sb(V)-treated groups at $p < 0.05$.

Effect of antimony administration on arsenic concentrations in blood and urine

Although arsenic is not recognized as an essential element in animals, it can be detected in the body at a substantial level. Accumulated arsenic in control experimental animals originates from their feeds. Indeed, arsenic was markedly accumulated in the whole blood and RBCs of rat (Figs. 3a and 3c). Arsenic concentrations in the whole blood and RBCs of hamster were much lower than those of rat, indicating that rat RBCs more preferably accumulated arsenic than hamster RBCs. These observations confirm the species difference in arsenic metabolism between rat and hamster, and are in agreement with a previous report (Shiobara *et al*., 2001).

Antimony administration significantly reduced arsenic concentrations in rat whole blood and RBCs (Figs. 3a and 3c). Because Sb was also accumulated in rat RBCs, Sb accumulation would competitively reduce arsenic accumulation in rat RBCs. Urinary arsenic excretion in rat was also reduced by the Sb treatment (Fig. 4). This suggested that Sb competed with arsenic transporters in renal tubules. On the other hand, no significant differences in plasma arsenic concentrations were noted between the control and the Sb-treated groups, between rat and hamster, and between the Sb(III)- and Sb(V)-treated groups (Fig. 3b).

As mentioned above, Sb accumulation in RBCs exhibited the same species difference as arsenic accumulation. According to previous literature, a chemical form of accumulated arsenic is dimethylated arsenic (Mandal *et al*., 2004; Rehman and Naranmandura, 2012; Suzuki *et al*., 2004). The ingredients of feed originating from marine biota are expected to contain low-toxicity organoarsenic compounds, such as arsenobetaine, arsenocholine, and arsenosugar (Hanaoka *et al*., 1988; Irvin and Irgolic, 1988; Larsen, 1995). When ingested, most orga-

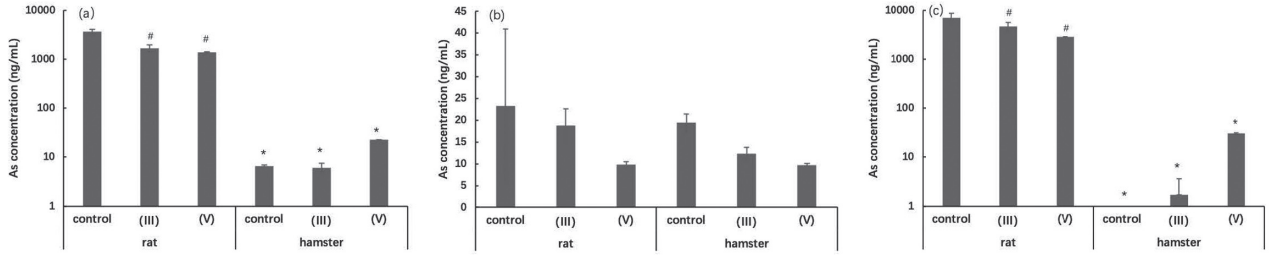

Fig. 3. Arsenic concentrations in whole blood (a), plasma (b), and red blood cells (c) of Wistar rat and Syrian hamster orally administered potassium antimony tartrate (Sb(III)) and potassium hexahydroxoantimonate (Sb(V)). Data are expressed as means ± S.D. of 4 samples. # indicates significant difference between control and Sb-treated groups at $p < 0.05$. * indicates significant difference between rat and hamster groups at $p < 0.05$.

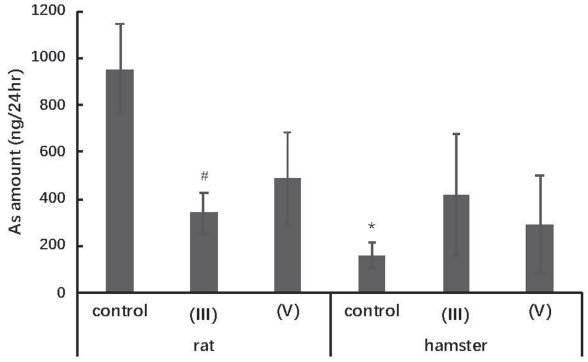

Fig. 4. Arsenic levels in urine of Wistar rat and Syrian hamster orally administered potassium antimony tartrate (Sb(III)) and potassium hexahydroxoantimonate (Sb(V)). Data are expressed as means ± S.D. of 4 samples. # indicates significant difference between control and Sb-treated groups at $p < 0.05$. * indicates significant difference between rat and hamster groups at $p < 0.05$.

noarsenic compounds are excreted in intact forms, and trace amounts are decomposed into dimethylated forms. On the other hand, it is reported that inorganic Sb is never methylated and excreted into urine in its intact inorganic form (Bailly *et al*., 1991; Kobayashi and Ogra, 2009). Therefore, it is possible that inorganic Sb and dimethylated arsenic behave similarly in the body.

Although Sb is widely used in our daily lives, its toxicity has remained unclear. Thus, more *in vivo* experiments should be conducted to reveal Sb toxicity. Because rat is highly tolerant to arsenic toxicity, it is an inappropriate animal model for studies of arsenic toxicity. Likewise, it is also an inappropriate animal model for studies of Sb toxicity for the same reason as arsenic.

In conclusion, tri- and pentavalent inorganic Sb showed species difference in their accumulation in RBCs. Rat RBCs more preferably accumulated Sb than hamster RBCs. Arsenic is accumulated in the dimethylated form, whereas Sb is probably accumulated in the inorganic form. Speciation analyses are needed to clarify further Sb metabolism in a future study.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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