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Acute toxicity and rapid excretion in urine of tetramethylarsonium salts found in some marine animals

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Acute toxicity in mice, and excretion in their urine, of tetramethylarsonium salts which are arsenic compounds found in some marine animals, were examined using synthetic tetramethylarsonium iodide. The oral, intraperitoneal and intravenous LD_{50} values of tetramethylarsonium iodide [$(CH_3)_4As^+I^-$] were determined to be 890, 175 and 82 mg kg^{-1}, respectively. When sublethal doses of tetramethylarsonium iodide were orally administered to mice, 53–58% of the arsenic administered was recovered in urine after 6 h and 65–81% after 72 h. High-performance liquid chromatography–inductively coupled plasma (HPLC–ICP) and fast atom bombardment mass spectrometric (FAB MS) analyses revealed that a tetramethylarsonium salt was the only arsenic compound excreted in urine. These results suggested that the major part of orally administered tetramethylarsonium iodide was absorbed from the gastrointestinal tract in mice and then rapidly excreted in urine without biotransformation.

Keywords: Arsenic, tetramethylarsonium salts, mice, acute toxicity, excretion, urine, biotransformation

INTRODUCTION

High levels of arsenic are contained in various marine organisms, chiefly as organic compounds. Since many marine organisms are utilized as important foodstuffs, it is necessary to elucidate the chemical structure of organoarsenicals in marine organisms and then examine their toxicity and metabolism in mammals. It has been established in the last decade that arsenobetaine [$(CH_3)_3As^+CH_2COO^-$] is the most prevalent organoarsenical in marine organisms. In a limited number of species, arsenosugars,[1–5] arsenocholine [$(CH_3)_3As^+CH_2CH_2OH$][6–9] and trimethylarsine oxide [$(CH_3)_3AsO$][10] have also been detected. Of these organoarsenicals, only arsenobetaine and arsenocholine have so far been studied with regard to their fate in mammals. Fortunately, arsenobetaine was shown to have no substantial acute toxicity; its LD_{50} (oral administration to mice) was over 10 g kg^{-1}.[11] When orally administered to mice, it was almost completely absorbed from the gastrointestinal tract and rapidly excreted in urine without biotransformation.[12] Intravenous administration also resulted in rapid excretion in the urine of mice, rats and rabbits. In the case of arsenocholine, although similar absorption and excretion patterns were observed, the major arsenical excreted in urine was not arsenocholine but arsenobetaine, suggesting the occurrence of biotransformation.[13]

Besides the organoarsenicals mentioned above, we recently detected tetramethylarsonium salts in the clam *Meretrix lusoria*,[14] the sea hare *Aplysia kurodai*[15] and the sea anemone *Parasicyonis actinostoloides*.[15] In relation to this, it is noticeable that tetramine (tetramethylammonium ion [$(CH_3)_4N^+$]), the nitrogenous analogue of the tetramethylarsonium ion, is known to be the causative compound of numerous intoxications in Japan due to ingestion of marine carnivorous gastropods, such as *Neptunea arthritica*.[16] Tetramine chloride [$(CH_3)_4N^+Cl^-$], which is the *in-vivo* existing form of tetramine, has been reported to be lethal to mice.[17] It could therefore be assumed that tetramethylarsonium salts might have acute toxicity in mammals. The present study was undertaken to ascertain this assumption. Furthermore, it was also useful to examine whether administered tetramethylarsonium salts can be rapidly excreted in urine. Since the precise salt form of the tetramethylarsonium ion in marine organisms is still unknown, synthetic tetramethylarsonium iodide was used as a model compound in this study.

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MATERIALS AND METHODS

Toxicity test

Acute toxicity was examined using male mice (ddY strain) weighing 20–25 g. Tetramethylarsonium iodide was dissolved in distilled water and administered into mice orally (p.o.), intraperitoneally (i.p.) or intravenously (i.v.). For each administration route, groups of eight mice were subjected to several doses and mortality was estimated after 1 h. LD_{50} values were calculated according to the method of Litchfield and Wilcoxon.[18] For comparison, p.o. and i.p. LD_{50} values of tetramine chloride were determined similarly.

Collection of urine following p.o. administration

Before administration, mice were kept without food and water for 12 h. A group of eight mice was administered p.o. with tetramethylarsonium iodide at a sublethal dose of 400 mg kg^{-1} and that of five mice at a dose of 100, 20 or 5 mg kg^{-1}. After administration, each group of mice was housed in a wire netting cage and given food and water *ad libitum*. Urine was collected on a filter paper spread under the cage at intervals indicated in Fig. 1. To avoid contamination, feces on the filter paper were removed as frequently as possible.

Determination of arsenicals in urine

The filter paper on which the urine was absorbed was cut into small pieces and extracted with methanol. The methanolic extract was evaporated to dryness and suspended in 5 or 10 cm^3 of 5% perchloric acid. After centrifugation, the supernatant was determined for arsenic with an inductively coupled argon plasma emission spectrometer (ICP; Jarrell Ash AtomComp Series 800). For the analysis of chemical forms of the arsenic excreted in urine, the supernatant, after being passed through a 0.45 μm membrane filter, was applied to a high-performance liquid chromatography (HPLC)–ICP system developed by Shiomi *et al.*[9] In brief, a column of Nucleosil 10SA (Nagel, 0.46 cm × 25 cm) was used with a 0.1 mol dm^{-3} pyridine–formic acid buffer (pH 3.1). The eluate was directly introduced into the nebulizer of the ICP and arsenic concentrations were recorded at 10 s intervals. Disodium arsenate, arsenobetaine, arsenocholine and tetramethylarsonium iodide were used as standard arsenicals.

As described below, only one arsenical was detected in each urine sample by HPLC–ICP. In order to purify this compound, a part of the supernatant prepared from the 0–6 h urine of the mice administered at 400 mg kg^{-1} was subjected to HPLC on Nucleosil 10SA with a 0.1 mol dm^{-3} pyridine–formic acid buffer (pH 3.1). The flow rate was maintained at 1 cm^3 min^{-1} and each 0.25 cm^3 fraction was

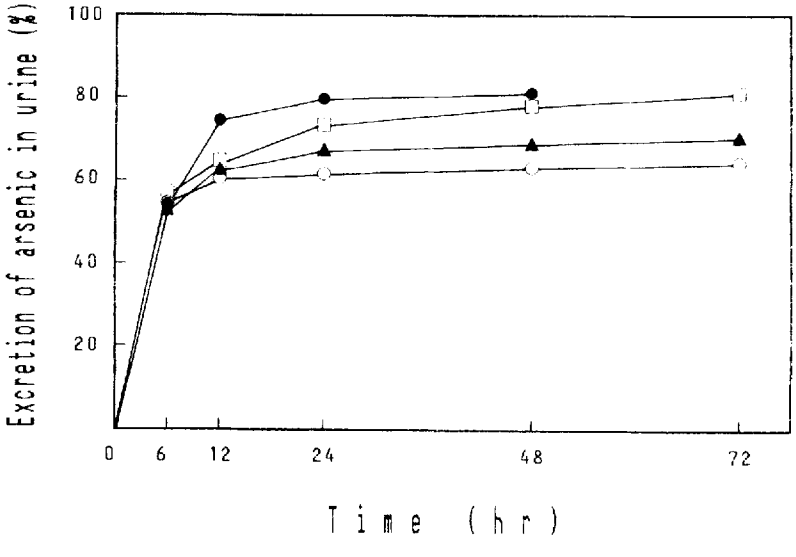

Figure 1 Excretion of arsenic in urine of mice following p.o. administration of tetramethylarsonium iodide. The administration doses are 400 mg kg^{-1} (•), 100 mg kg^{-1} (○), 20 mg kg^{-1} (▲) and 5 mg kg^{-1} (□).

collected. Arsenic-containing fractions (fractions 30–35) were combined and evaporated to dryness. The dried material was dissolved in a 0.02 mol dm^{-3} pyridine–formic acid buffer (pH 3.1) and again subjected to HPLC with the same buffer. Fractions of 0.5 cm^3 were collected at a flow rate of 1 cm^3 min^{-1}. Arsenic-containing fractions (fractions 36–42) were combined and evaporated to dryness. The fast atom bombardment (FAB) mass spectrum of the purified arsenical was measured with a JEOL JMS-DX 300 double-focusing mass spectrometer. Glycerol was used as a matrix on the target probe. Ionization of the sample was achieved by xenon atoms at 6 kV.

RESULTS

Acute toxicity

Tetramethylarsonium iodide, like tetramine chloride, exhibited acute toxicity in mice. The LD_{50} values of both compounds are summarized in Table 1. Since it is more convenient to compare the LD_{50} values of tetramethylarsonium ion with those of tetramine in the same salt form, the values of tetramethylarsonium chloride calculated from those of iodide are listed together in the table. The results reveal that tetramethylarsonium salts are clearly less toxic than the corresponding nitrogenous compound, tetramine chloride. Tetramethylarsonium chloride was about 24 times less toxic than tetramine chloride on p.o. administration and about 7 times less toxic on i.p. administration.

Immediately after p.o. or i.p. administration of tetramethylarsonium iodide at lethal doses, mice exhibited an acceleration of spontaneous motility which was frequently accompanied by grooming. The spontaneous motility was inhibited in a few minutes and instead vasodilation and respiratory depression appeared, followed by mild ataxia with tremor. These symptoms continued for a while. Finally the mice showed severe tremor with tonic convulsion and salivation and died of respiration arrest after several fits of gasping, usually within 40 min. No mice survived over 1 h. In the case of p.o. or i.p. administration of tetramine chloride at lethal doses, essentially the same symptoms were observed in mice, which also died within 40 min. On the other hand, i.v. administration of tetramethylarsonium iodide at lethal doses instantly caused severe tremor in mice, with tonic convulsion but without salivation, and killed them within 10 min. At sublethal doses of tetramethylarsonium iodide or tetramine chloride, symptoms such as ataxia and tremor were induced in mice to some extent but ceased in a short time; the mice were completely restored after 30 min.

Excretion of arsenic in urine

In Fig. 1 the excretion of arsenic in urine of mice following p.o. administration of tetramethylarsonium iodide is shown. The excretion pattern of arsenic appeared to be almost independent of the administration dose in the range 5–400 mg kg^{-1}. The arsenic given was rapidly excreted in urine; 53–58% of the arsenic was recovered in the 0–6 h urine. Though to a lesser extent, the excretion of arsenic in urine continued after 6 h. The recovery of arsenic in urine reached about 81% after 48 h at 400 mg kg^{-1} and 65–81% after 72 h in the other cases.

Identification of arsenicals excreted in urine

When analyzed by HPLC–ICP, all of the 0–6 h urine samples afforded a single arsenic peak. As a typical example, the result of the 0–6 h urine at 400 mg kg^{-1} is illustrated in Fig. 2B. The retention time of the arsenical in urine coincided well with that of tetramethylarsonium iodide (see Fig. 2A). Figure 2C shows the result of the 24–48 h urine at 400 mg kg^{-1}. In this case also, only one arsenic peak

Table 1 LD_{50} values of tetramethylarsonium salts and tetramine chloride

Administration route	LD_{50} value (mg kg^{-1})		
	Tetramethylarsonium iodide	Tetramethylarsonium chloride[a]	Tetramine chloride
p.o.	890 (810–980)[b]	580 (530–640)	24 (21–27)
i.p.	175 (164–186)	114 (107–121)	16 (15–17)
i.v.	82 (67–101)	53 (44–66)	

[a] Calculation from the values of tetramethylarsonium iodide on the basis of the different molar masses. [b] 95% confidence intervals are indicated in parentheses.

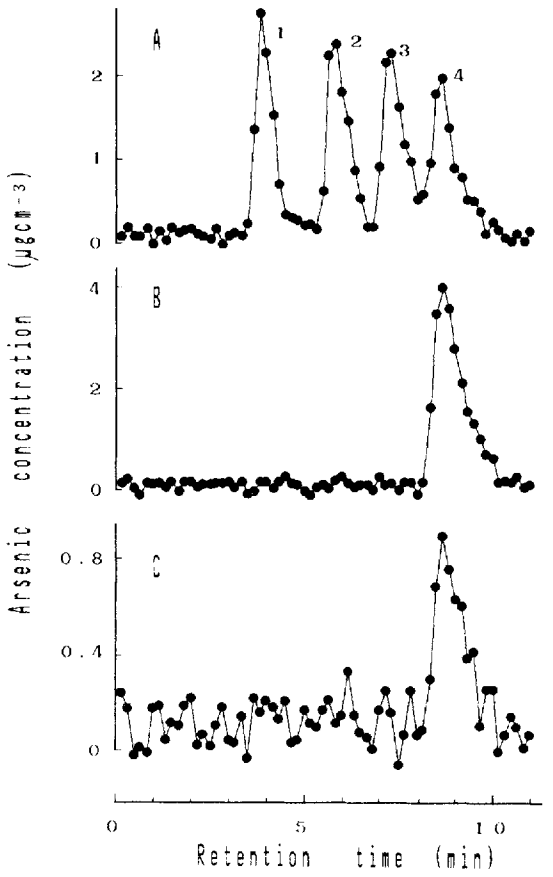

Figure 2 HPLC of standard arsenicals (A), the 0–6 h urine at 400 mg kg^{-1} (B) and the 24–48 h urine at 400 mg kg^{-1} (C) monitored by ICP. Column, Nucleosil 10SA (0.46 cm × 25 cm); solvent, 0.1 mol dm^{-3} pyridine–formic acid buffer (pH 3.1); flow rate, 1 cm^3 min^{-1}. The standard arsenicals are: 1, disodium arsenate; 2, arsenobetaine; 3, arsenocholine; and 4, tetramethylarsonium iodide.

was observed at the same retention time as tetramethylarsonium iodide. Thus, it was assumed that irrespective of the time elapsed after administration, the only arsenical excreted in urine was a tetramethylarsonium salt. Definite evidence for the assumption was obtained by FAB MS analysis. As shown in Fig. 3, the spectrum of the purified arsenical gave a peak at *m/e* 135, which is characteristic for tetramethylarsonium salts.[14,15]

DISCUSSION

Tetramethylarsonium salts were demonstrated to have acute toxicity in mice similar to that of tetramine chloride. There was no difference in the symptoms of mice between tetramethylarsonium iodide and tetramine chloride, although the former was apparently less toxic than the latter, indicating that both compounds manifest their acute toxicity by the same mode of action. It should be pointed out that tetramethylarsonium salts are significantly higher in toxicity than arsenobetaine, the major arsenic species in marine organisms, which has no substantial acute toxicity in mice.[11] This fact for $(CH_3)_4As^+I^-$ seems to go against the generalization that the toxicity of inorganic arsenicals is almost eliminated by their methylation in marine organisms. In this respect, although the distribution of tetramethylarsonium salts in marine organisms may be much more limited than that of arsenobetaine, they are no doubt of greater toxicological importance. Judging from the concentration in the three species of marine animals which have been shown to contain tetramethylarsonium salts,[14,15] the acute toxicity estimated in this study does not neces-

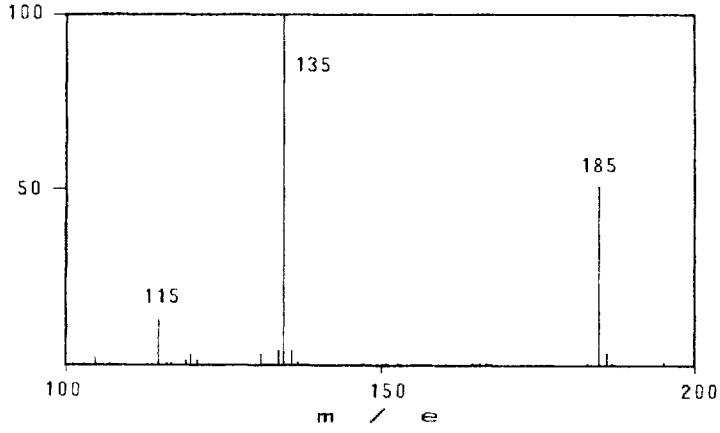

Figure 3 FAB mass spectrum of the purified arsenical. The peaks at *m/e* 115, 135 and 185 are assignable to $(glycerol + Na)^+$, $(CH_3)_4As^+$ and $(2 \times glycerol + H)^+$, respectively.

sarily imply that tetramethylarsonium salts in marine organisms are seriously hazardous to human health. However, it is generally known that humans are much more sensitive to the toxic effects of arsenic than are rodents.[19] Thus, further study is needed to estimate the acute toxicity of tetramethylarsonium salts in mammals other than mice. At the same time the distribution and concentration of tetramethylarsonium salts in marine organisms, especially in edible species, should be confirmed, as they might have been overlooked before their recent discovery.

The results of the excretion experiments strongly suggest that, following p.o. administration, the major part of tetramethylarsonium iodide was absorbed from the gastrointestinal tract and then rapidly excreted in urine via the kidney. The rapid excretion in urine may account for the fact that the symptoms in mice induced by sublethal doses of tetramethylarsonium iodide cease in a very short time. In addition, the absence of substantial biotransformation of tetramethylarsonium iodide was also evidenced by HPLC–ICP and FAB MS analyses of urine samples. The precise salt form of tetramethylarsonium ion excreted in urine still remains unsolved. However, even though tetramethylarsonium iodide could be transformed to other salt forms, such biotransformation is not likely to be significant in a toxicological sense.

Tetramethylarsonium salts may be more comparable with arsenobetaine rather than with arsenocholine, not only in that they are excreted in urine at high concentrations in a short period but also in that they do not undergo biotransformation. It was reported for arsenobetaine that about 76% of the p.o.-administered arsenic appeared in mouse urine without biotransformation within 24 h, and as much as 98% within 72 h.[12] In contrast, following p.o. administration in mice, arsenocholine was transformed to arsenobetaine to a large extent.[13] Also, the excretion rate in urine of the administered arsenic was relatively low as compared with the case of arsenobetaine; about 22% of the arsenic was recovered within 24 h and about 64% within 72 h. It is worth mentioning that the recovery of tetramethylarsonium salts reported in this study must be the minimum value, since at least a small portion of urine could not be recovered due to absorption by feces as well as adhesion to the cage. However, the recovery of tetramethylarsonium salts in urine after 72 h does not appear to match that of arsenobetaine. It is possible that a small amount of the tetramethylarsonium salt is eliminated in feces and/or retained in the body. In order to obtain a more detailed toxicological knowledge of tetramethylarsonium salts, their elimination in feces and retention in the body as well as their excretion in urine should be investigated using mammals including mice.

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