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Acute toxicity and metabolism of arsenocholine in mice

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The acute toxicity of arsenocholine was examined in mice by oral administration and intravenous injection. The LD_{50} values of arsenocholine were 6.5 g kg^{-1} for oral administration and 187 mg kg^{-1} for intravenous injection. Decreases of respiration and spontaneous motility were observed in the mice dosed orally at 12 g kg^{-1}. The animals exhibited ataxia and finally showed paralysis of the hind legs within 20 min of administration. When arsenocholine was administered orally to mice at 5 or 50 mg As kg^{-1}, the greater part of the arsenic administered was recovered in urine within 96 h. The metabolite of arsenocholine in urine was identified as arsenobetaine by high-performance liquid chromatography–inductively coupled plasma emission spectrometry (HPLC ICP) and fast atom bombardment mass spectrometry (FAB MS). These results suggested that the major part of orally administered arsenocholine was absorbed from the gastrointestinal tract in mice and then rapidly excreted in urine with biotransformation.

Keywords: Arsenocholine, arsenobetaine, acute toxicity, LD_{50}, excretion, metabolism

INTRODUCTION

It is known that marine organisms contain high levels of arsenic, and it has been shown that the arsenic compounds in many marine organisms are water-soluble organoarsenic compounds. Arsenobetaine, trimethyl(carboxymethyl)arsonium zwitterion, is a major water-soluble organoarsenic compound. It is found only in marine animals and therefore seems to be the final metabolite in the arsenic cycle of marine ecosystems.[1,2] Other organoarsenic compounds found in marine organisms are arsenocholine,[3–7] arseno-sugars,[8–11] tetramethylarsonium[12,13] salts and lipid-soluble arsenic compounds.[14]

Generally, arsenicals have high potential toxicity. It is certain that a large number of people consuming a variety of marine food products are daily exposed to many arsenic compounds, especially organoarsenic ones. There is a need to investigate the toxicological properties of organoarsenic compounds in marine organisms. We have reported that arsenobetaine administered orally to mice at a dose of 10 g kg^{-1} body weight did not cause any toxic symptoms and that it was excreted rapidly in urine without biotransformation.[15] Rapid excretion of arsenobetaine was also established in hamsters.[16]

Arsenocholine, trimethyl(2-hydroxyethyl)-arsonium cation, detected at low levels in shrimp[3–6] and conch,[7] is thought to be a possible candidate as the precursor of arsenobetaine in the marine food chain.[1,2,4] Marafante *et al.* examined the metabolism of arsenocholine in experimental animals and reported that it was converted to arsenobetaine and rapidly excreted in urine.[17] There is no report on toxicological studies with arsenocholine except for a study of prenatal toxicity (not found) to rat embryos.[18]

In this paper we report the acute toxicity and metabolism of arsenocholine in mice in order to elucidate the toxicological properties of arsenocholine from the viewpoint of food hygiene.

EXPERIMENTAL

Chemicals

Arsenocholine [trimethyl(2-hydroxyethyl)arsonium bromide] was prepared according to the procedure described by Saaman.[19] Arsenobetaine was synthesized by the method of Cannon *et al.*[20]

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Animals

Five-week-old male ddY mice (Shizuoka Laboratory Animal Co., Japan) weighing 20–25 g were used for acute toxicity and excretion studies after quarantining for one week in a conditioning room at 23 ± 2 °C and at 55 ± 5% relative humidity. Pelleted dry diet (CE2: Clea Japan Inc., Japan) and tap-water were fed *ad lib.*

Median lethal dose

Only in the case of oral administration mice were kept without diet and water for 12 h or more before administration. Seven or ten animals were used for each dose. Arsenocholine was dissolved in distilled water for oral administration and in physiological sodium chloride solution for intravenous injection. The solutions of arsenocholine were orally administered with a cannula to the group of ten animals or intravenously injected to the group of seven animals.

The mice were observed for symptoms at all times for the initial 5 h following administration and subsequently every 1 h up to 24 h and then every day for seven days. The LD_{50} values were calculated statistically by the probit method.

Excretion of arsenic in urine and feces

Arsenocholine was administered orally at the sublethal dose of 5 mg As kg^{-1} or 50 mg As kg^{-1} to two groups of three mice. After administration, each group of mice was housed in a metabolic cage and given food and water *ad lib.* Urine and feces were collected at intervals of 24 h.

Total arsenic in the collected urine was measured directly with an inductively coupled argon plasma emission spectrometer (ICP; Jarrell Ash AtomComp Series 800). The feces (1–5 g) were digested on a hot plate with a mixture of nitric, perchloric and sulfuric acids (25:5:0.5) until dense fumes of sulphur trioxide appeared, and then determined for arsenic with the ICP. The conditions for the ICP system were as follows: wavelength, 193.7 nm; integration time, 20 s; nebulizer, 1.4 cm^3 min^{-1}.

Determination of arsenicals in urine

Analysis by HPLC ICP

To the collected urine in a metabolic cage was added an equal volume of 10% perchloric acid. The suspension was centrifuged and the supernatant was passed through a 0.45-μm membrane filter and applied to a high-performance liquid chromatograph connected to the ICP (HPLC ICP). The HPLC conditions are as follows: column, strong ion exchange (acidic cation) Nucleosil 10SA (Nagel, 0.46 cm × 25 cm); mobile phase, 0.1 mol dm^{-3} pyridine–formic acid buffer (pH 3.1); flow rate, 1.0 cm^3 min^{-1}; temperature, ambient. The eluate was introduced directly into the nebulizer of the ICP and arsenic concentrations were recorded at 10 s intervals (integration time here = 5 s). Disodium arsenate, arsenobetaine, arsenocholine bromide and tetramethylarsonium iodide were used as standard arsenicals.

Analysis by FAB mass spectrometry

In a separate experiment, a group of ten mice was administered with arsenocholine at a dose of 500 mg kg^{-1}. The mice were housed in a wire netting cage and given food and tap-water *ad lib.* Urine was collected on a filter paper spread under the cage for periods of 24 h. Feces on the filter paper were removed as frequently as possible to avoid contamination. The filter paper on which the urine was absorbed was cut into small pieces and extracted with methanol. In order to purify arsenicals in urine, the extract was subjected to column chromatography on Dowex 50w × 8 (H^+ form), Dowex 2 × 8 (OH^- form), Sephadex LH20, and active carbon columns as described by Hanaoka and Tagawa.[21] The purified arsenical was measured with a JEOL JMS-DX 300 mass spectrometer equipped with a fast atom bombardment (FAB) ion source and xenon atoms at 6 keV as reported by Kaise *et al.*[22]

RESULTS AND DISCUSSION

Acute toxicity

The mice orally administered with arsenocholine at a lethal dose of 12 g kg^{-1} exhibited an acceleration of spontaneous motility and frequently reacted sensitively under external stimulus after 5 min. Respiratory depression appeared and the spontaneous motility was inhibited after 7–10 min, followed by ataxia. The animals showed a cyanosis with respiratory depression after 10 min and subsequently had diarrhoea. Furthermore, the mice showed ataxia followed by paralysis of the hind legs. Finally they showed twitching and disappearance of balance with writhing of the

Table 1 Acute toxic symptoms of mice after oral administration of arsenocholine at a dose of 12.0 g kg^{-1}

	Time after dosing: 0–15 min	1 h	5 h	24 h
Spontaneous	⇅	↓	↓	↓
Grooming	–	–	–	–
Restlessness	+	–	–	–
Fighting	–	–	–	–
Squeaking	–	–	–	–
Sleeping	–	–	–	–
Nociceptive reflex	–	–	–	–
Startle reflex	↑	↑	–	–
Balance	–	–	–	–
Body position	–	–	–	–
Ataxia	+	+	–	–
Straub's tail reaction	–	–	–	–
Muscle tone	–	–	–	–
Tremor	–	–	–	–
Twitch	+	+	–	–
Tonic convulsion	–	–	–	–
Clonic convulsion	–	–	–	–
Salivation	–	–	–	–
Urination	–	–	–	–
Defecation	+	+	+	+
Piloerection	–	–	–	–
Skin color	↓	⇊	⇊	⇊
Heart rate	–	–	–	–
Respiratory rate	↓	⇊	⇊	⇊
Gasping	–	–	–	–
Death	0	4	4	2

Key: – = normal; + = appearance; ↑ = acceleration; ↓ = depression or decrease.

limbs, and died of respiratory arrest after several fits of gasping. Four out of ten mice died within 1 h and the rest within 24 h.

Autopsy revealed a slight congestion of the duodenum and small intestine. These symptoms are summarized in Table 1. Intravenous injection of lethal doses induced essentially the same symptoms in mice as described above but the mice were all dead within 1 h.

The mortality of mice at the LD_{50} is shown in Table 2.

The LD_{50} values of arsenocholine were 6.5 g kg^{-1} (5.8–7.2 g: 95% confidence limits) for oral administration and 187 mg kg^{-1} (177–200 mg) for intravenous injection, i.e. a 30-fold difference between the administration routes. It was thought that arsenocholine affected the nervous systems directly and produced acute toxic symptoms in the case of intravenous injection.

The oral LD_{50} value of arsenobetaine has been reported to be above 10 g kg^{-1}.[15] Although arsenocholine is slightly more toxic than arsenobetaine, its oral toxicity appears to be insignificant. Arsenocholine was reported to have no embryotoxicity or embryolethality by Irvin and Irgolic.[18]

Excretion of arsenic

Figure 1 shows the excretion of arsenicals in urine and feces of mice after oral administration of arsenocholine at doses of 5 mg As kg^{-1}.

The arsenic administered was rapidly excreted in urine; 52% of the administered arsenic was recovered in the 0–24 h urine. The recovery of arsenic in urine reached about 71% after 48 h and 78% after 72 h. The excretion of arsenic in feces

Table 2 Dose and mortality of arsenocholine

Dose (mg kg^{-1})	Number of deaths 0–1 h	1–5 h	5 h–1 day	1–5 days	5–7 days	Mortality*	LD_{50} (95% confidence limits)
Intravenous							
220	2	0	0	0	0	2/2	
199	4	0	0	0	0	4/7	
182	3	0	0	0	0	3/7	187 mg kg^{-1} (177–200 mg)
165	1	0	0	0	0	1/7	
150	0	0	0	0	0	0/7	
Oral							
12000	4	4	2	0	0	10/10	
10000	3	4	2	0	0	9/10	
8330	2	3	3	0	0	8/10	6.5 g kg^{-1}
6940	0	1	4	2	0	7/10	(5.8–7.2 g)
5790	0	0	1	3	0	4/10	
4820	0	0	0	0	0	0/10	

* Numbers of animals out of total.

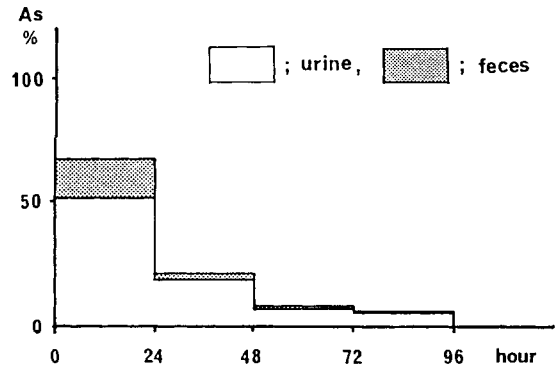

Figure 1 Excretion of arsenic in the urine and feces of mice after oral administration of arsenocholine.

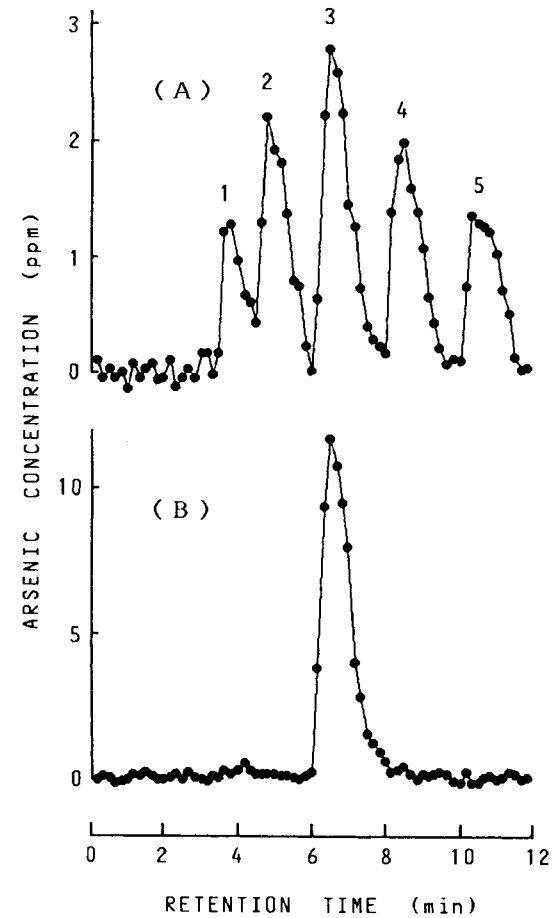

Figure 2 High-performance liquid chromatograms of standard arsenicals (A) and 0–24 h urine from animals dosed at 50 mg As kg^{-1} (B) monitored by ICP. Column, Nucleosil 10SA (0.46 cm × 25 cm); mobile phase, 0.1 mol dm^{-3} pyridine–formic acid buffer (pH 3.1); flow rate, 1 cm^3 min^{-1}. Standard arsenicals: 1, arsenate; 2, dimethylarsinic acid; 3, arsenobetaine; 4, arsenocholine; 5, tetramethylarsonium iodide.

after oral administration was 18% of the dose during 96 h. Almost all of the dose of arsenic at 5 mg As kg^{-1} was excreted in the urine and feces within four days. The urinary excretion of arsenic during the 96 h after administration of arsenocholine was 83% of the administered dose. The animals orally administered at dose of 50 mg As kg^{-1} showed the same pattern of the excretion in the urine and feces as well as dose of 5 mg As kg^{-1}. The urinary excretion of arsenic reached about 40% after 24 h, 50% after 48 h and 61% after 96 h and the arsenic was excreted in the feces 24% after 24 h and 25% after 96 h.

These results are essentially the same as those of Marafante *et al.*[17] and suggested that the major part of orally administered arsenocholine was absorbed from the gastro-intestinal tract in mice and then rapidly excreted in urine via the kidney.

Identification of arsenical excreted in urine

Analysis by HPLC ICP showed that the arsenical excreted in urine was arsenobetaine regardless of the dose administered and the time elapsed after administration. As a typical result, the chromatogram of the arsenical recovered in the 0–24 h urine after administration of 50 mg As kg^{-1} is illustrated in Fig. 2(B). The retention time of the arsenical agreed well with that of arsenobetaine [see Fig. 2(A)].

Further evidence for the excretion of arsenobetaine in urine was obtained by FAB mass-spectrometric analysis. The arsenical purified by chromatographic procedures gave a molecular ion peak at m/z 179 in the FAB mass spectrum. The mass spectrum was essentially identical with that of synthetic arsenobetaine.[15] The FAB mass spectrum of the metabolite in urine is shown in Fig. 3.

Marafante *et al.* reported that arsenobetaine was the main metabolite found in ultrafiltered plasma and cytosols of tissues and urines of rabbits and mice after administration of arsenocholine from the behavior in ion-exchange chromatography.[17] We have confirmed their conclusion by means of HPLC ICP and FAB MS.

In conclusion, arsenocholine has no significant oral toxicity in mice worth consideration. Also, the major part of orally administered arsenocholine was absorbed from the gastro-intestinal tract in mice, converted to arsenobetaine which had less toxicity and rapidly excreted in urine.

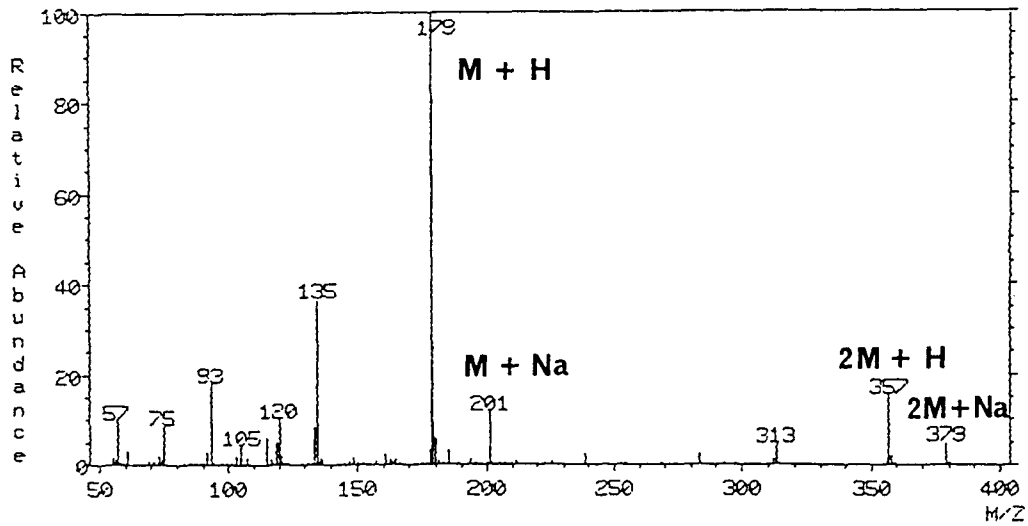

Figure 3 FAB MS spectrum of metabolite in the urine of mice after oral administration of arsenocholine at a dose of 500 mg kg^{-1}. G, Glycerine; M, Arsenobetaine; Na, Sodium.

Although arsenobetaine is present in some marine animals, it seems to pose no serious problem from the view-point of food hygiene.

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