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A comparative study on acute toxicity of methylarsonic acid, dimethylarsinic acid and trimethylarsine oxide in mice

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The acute toxicity of methylarsonic acid, $CH_3AsO(OH)_2$ (MAA), dimethylarsininc acid, $(CH_3)_2AsO(OH)$ (DMAA), and trimethylarsine oxide, $(CH_3)_3AsO$ (TMAO), were examined in mice with oral administration.

The LD_{50} values of MAA, DMAA and TMAO were 1.8, 1.2 and 10.6 g kg^{-1} respectively. The toxicity of MAA and DMAA was very much lower than that for inorganic arsenic compounds. It was shown that TMAO has a similar acute toxicity to arsenobetaine. On the other hand, when the mice were administered 14.4 g kg^{-1} of TMAO once only orally, a garlic-like odor (trimethylarsine, $(CH_3)_3As$) was definitely detectable in the exhalation of the animals by the human olfactory sense within about a few minutes.

Keywords: Methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide, arsenobetaine, trimethylarsine, acute toxicity, LD_{50}

INTRODUCTION

Generally inorganic arsenic compounds have high toxicity; arsenite particularly has the highest potential. Studies in human and experimental animals[1–4] have shown that methylarsonic acid (MAA) and dimethylarsinic acid (DMAA) are metabolites of inorganic arsenic. DMAA is at high concentrations in seaweeds, and humans take DMAA into the body by eating seafoods. On the other hand, the chemicals containing MAA or DMAA are used as arsenic agrochemicals. Recently, Norin *et al.*[5] reported the detection of an extremely small amount of trimethylarsine oxide (TMAO) in some fish. On the other hand, Marafante *et al.*[6] reported that the administration of DMAA to experimental animals resulted in the formation of TMAO *in vivo*. It is thought that TMAO is a intermediary compound between trimethylarsine and arsenobetaine, while there are few reports on the study of the behaviour and toxicological properties of TMAO *in vivo*.

It is thought the toxicological effects of these arsenic compounds depended on their chemical speciation and chemical structures such as differences in the number of methyl substituents on the arsenic atom. Whilst the metabolism of methylated arsenic compounds has been investigated in experimental animals, not much is known about the acute toxicity of TMAO.

The present study was undertaken to elucidate the toxicological properties of methylated arsenic compounds and describes the acute toxicity of MAA, DMAA and TMAO in experimental animals.

EXPERIMENTAL

Chemicals

Methylarsonic acid (MAA; analytical grade) was obtained from Trichemical Co., Japan, dimethylarsinic

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acid (DMAA; analytical grade) was purchased from Wako Pure Chemical Co., Japan, and trimethylarsine oxide (TMAO) was synthesized from trimethylarsine (Trichemical Co., Japan) using hydrogen peroxide according to the method of Kaise *et al.*[7]

Animals

Five-week-old male ddY mice (Shizuoka Laboratory Animal Co., Japan) were used after quarantining for one week in a conditioning room at $23 \pm 2°C$ and relative humidity $55 \pm 5\%$. Pelleted dry diet (CE2: Clea Japan Inc., Japan) and tap-water were fed *ad lib.*

Median lethal dose

Mice weighing 24–28 g were used in groups containing ten animals. MAA, DMAA and TMAO were dissolved with distilled water to a concentration of 10% w/w, 9% w/w and 50% w/w respectively, and the solutions of arsenic compounds were orally administered to the animals with a cannula at the following doses. MAA: 1.563, 1.652, 1.818, 2.000 and 2.200 g kg^{-1}. DMAA: 0.720, 0.900, 1.125, 1.406 and 1.757 g kg^{-1}. TMAO: 8.33, 10.00, 12.00 and 14.40 g kg^{-1}.

The poisoning symptoms were observed at all times after 5 h following administration and subsequent observations were made at intervals of 1 h until 24 h and on every day for seven days. The LD_{50} values were statistically calculated by the probit method.

RESULTS AND DISCUSSION

Acute toxic symptoms

In the group administered MAA at the lethal dose of 2.2 g kg^{-1}, the animals showed a decrease of respiration and spontaneous motility after 5–10 min; these symptoms continued through the experiment. Finally, mice died of respiration arrest following a few gasps after 6–12 h. The animals in other groups were also observed as having the same symptoms.

In the group administered DMAA at the lethal dose of 1.8 g kg^{-1}, the acute toxic symptoms were depression of spontaneous motility and decrease of respiration after 10–45 min. From 45 min to 1.5 h after administration, an acceleration of startle reflex and spontaneous motility was observed, and then the spontaneous motility was gradually depressed and the respiration was decreased followed by ataxia after 1.5 h. The animals died of respiration arrest following a few gasps after 6–12 h.

In the group administered TMAO at the lethal dose of 14.4 g kg^{-1}, a garlic-like odor was definitely smelled in the exhalation of the animals after 2–3 min, and the odor continued for a few hours. It was confirmed by GC MS that the odorous substance was trimethylarsine in a previous study.[8] The animals exhibited irritability, and subsequently ataxia and respiratory depression, followed by acceleration of spontaneous motility, and they occasionally showed startle motility. Finally, the animals showed paralysis of the hind legs after 20–40 min, and two mice died of respiration arrest within 1 h (in the first cases of death) and the rest died within 24 h.

More than half of the animals in the three groups administered lethal doses of MAA, DMAA and TMAO had 2–4 episodes of diarrhoea for an hour, but the only pathological finding was a slight congestion of the small intestine. These symptoms and mortalities were summarized in Table 1.

Acute toxicity

Figure 1 and Table 2 show the dose–mortality curves and LD_{50} values (95% confidence limits) of MMA, DMAA and TMAO.

The LD_{50} values of MAA and DMAA were 1.8 (1.7–1.9) and 1.2 (1.0–1.3) g kg^{-1}, respectively. In our previous paper,[9] the LD_{50} value of arsenic trioxide was found to be 34.5 mg kg^{-1} in mice and hence this arsenic form was extremely toxic. The toxicity of DMAA was slightly higher than that of MAA in the present study, whereas the order to toxic potency (LD_{50}) in rats from the data of NIOSH[10] were as follows: sodium arsenite (41 mg kg^{-1}) > monosodium methylarsonate (0.7 g kg^{-1}) > sodium dimethylarsinate (2.6 g kg^{-1}).

The LD_{50} value of TMAO was 10.6 (9.4–11.5) g kg^{-1} in mice. From these results, the LD_{50} values of DMAA and MAA against mice were about 30–50-fold higher than the LD_{50} value of arsenic trioxide and that of TMAO was about 300-fold higher than that.

TMAO is a trimethylarsenic compound, but it cannot be thought to be an arsenic compound occurring abundantly in the natural environment, e.g. in fish and shellfish, and perhaps also in mammals.

Table 1 Acute toxic symptoms of methylated arsenic compounds in mice
Key: —, normal; +, appearance; ↑, acceleration; ↓, depression or decrease

	Methylarsonic acid (MAA) (2.2 g kg^{-1} administered)				Dimethylarsinc acid (DMAA) (1.757 g kg^{-1} administered)				Trimethylarsine oxide (TMAO) (14.4 g kg^{-1} administered)			
	0–15 min	0–1 h	0–5 h	0–24 h	0–15 min	0–1 h	0–5 h	0–24 h	0–15 min	0–1 h	0–5 h	0–24 h
Spontaneous motility	↓	↓	↓	↓	↓	↑↑	↓	↓	↑	↑	↑	↑
Grooming	—	—	—	—	—	—	—	—	—	—	—	—
Restlessness	+	—	—	—	+	—	—	—	—	—	—	—
Fighting	—	—	—	—	—	—	—	—	—	—	—	—
Squeaking	—	—	—	—	—	—	—	—	—	—	—	—
Sleeping	—	—	—	—	—	—	—	—	—	—	—	—
Nociceptive reflex	—	—	—	—	—	—	—	—	—	—	—	—
Startle reflex	—	—	—	—	—	+	—	—	—	+	+	—
Righting	—	—	—	—	—	—	—	—	—	—	—	—
Body position	—	—	—	—	—	—	—	—	—	—	—	—
Ataxia	—	—	—	—	—	—	+	+	—	+	+	+
Straub's tail reaction	—	—	—	—	—	—	—	—	—	—	—	—
Muscle tonus	—	—	—	—	—	—	—	—	—	—	—	—
Tremor	—	—	—	—	—	—	—	—	—	—	—	—
Twitch	—	—	—	—	—	—	—	—	—	—	—	—
Tonic convulsion	—	—	—	—	—	—	—	—	—	—	—	—
Clonic convulsion	—	—	—	—	—	—	—	—	—	—	—	—
Salivation	—	—	—	—	—	—	—	—	—	—	—	—
Urination	—	—	—	—	—	—	—	—	—	—	—	—
Defecation (diarrhoea)	—	—	+	+	—	+	+	+	—	+	+	+
Piloerection	—	—	—	—	—	—	—	—	—	—	—	—
Skin color	↓	↓↓	↓↓	↓↓	—	—	—	—	—	—	—	—
Heart rate	—	—	—	—	—	—	—	—	—	—	—	—
Respiratory rate	↓	↓↓	↓↓	↓↓	↓	↓	↓	↓	↓	↓	↓	↓
Gasping	—	—	—	—	—	—	—	—	—	—	—	—
Odor of exhalation	—	—	—	—	—	+	+	—	+	+	+	+
Death	0	0	0	10	0	0	0	10	0	2	7	10

Table 2 Dose and mortality of methylated arsenic compounds

Dose ($g\ kg^{-1}$)	No. of treated mice	No. of deaths						Mortality	LD_{50}($g\ kg^{-1}$) (95% confidence limits)	Mean body weight (g)	
		0–15 min	15 min–1 h	1 h–5 h	5 h–1 day	1 day–5 days	5 days–7 days			Initial	Final
Methylarsonic acid (MAA)											
1.563	10	0	0	0	0	0	0	0/10		24.4	27.6
1.652	10	0	0	0	0	3	0	3/10		24.3	27.3
1.818	10	0	0	0	1	3	0	4/10	1.8 (1.7–1.9)	24.3	27.7
2.000	10	0	0	0	7	2	0	9/10		24.2	28.0
2.200	10	0	0	0	10	—	—	10/10		24.8	27.7
Dimethylarsinic acid (DMAA)											
0.720	10	0	0	0	0	0	0	0/10		24.8	27.8
0.900	10	0	0	0	0	1	0	1/10		25.2	27.9
1.125	10	0	0	0	2	1	0	3/10	1.2 (1.0–1.3)	26.8	27.8
1.406	10	0	0	0	7	2	0	9/10		26.6	27.6
1.757	10	0	0	0	10	—	—	10/10		24.7	27.7
Trimethylarsine oxide (TMAO)											
8.33	10	0	0	0	0	0	0	0/10		23.7	26.4
10.00	10	0	0	0	1	2	0	3/10	10.6 (9.4–11.5)	23.5	26.5
12.00	10	0	0	3	4	2	0	9/10		23.6	26.1
14.40	10	0	2	5	3	—	—	10/10		25.3	26.5

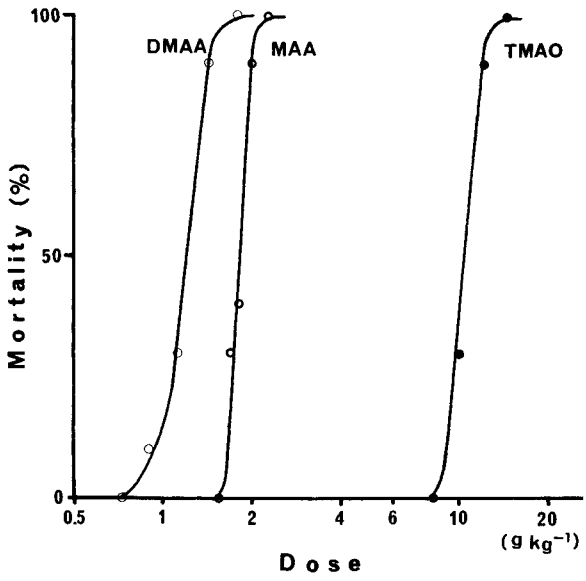

Figure 1 Dose–mortality curves of methylarsonic acid (MAA), dimethylarsinic acid (DMAA) and trimethylarsine oxide (TMAO)

Arsenobetaine $[(CH_3)_3As^+CH_2COO^-]$, which is also a trimethylarsenic compound, was estimated as a non-toxic arsenical ($LD_{50} > 10$ g kg^{-1}) in our paper,[9] and it has been observed to be widely distributed in many marine animals.[11–18] In the present study, it has been demonstrated that TMAO is similar in acute toxicity to arsenobetaine. Also, the decrease of arsenical toxicity for animals seemed to depend on the increase in the number of methyl substituents on the arsenic atom.

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