Bull. Environ. Contam. Toxicol. (1986) 36:350–355

Environmental Contamination and Toxicology

Metabolism and Excretion of Orally Administered Arsenobetaine in the Hamster

Hiroshi Yamauchi,[1] Toshikazu Kaise,[2] and Yukio Yamamura

[1]Department of Public Health, St. Marianna University School of Medicine, 2095, Sugao, Miyamae-ku, Kawasaki 213, Japan and [2]Department of Food and Drug Science, Kanagawa Prefectural Public Health Laboratory, 52-2, Nakao-cho, Asahi-ku, Yokohama 241, Japan

Arsenobetaine, one of the trimethylarsenic compounds (TMA), occurs abundantly in seafoods (Edmonds et al 1977; Cannon et al. 1981). To the Japanese who favor seafoods, it is of the utmost importance to know the metabolism, *in vivo* accumulation and excretion of this compound. However, there has been no report from Japan on the impairment of health due to the arsenic contained in seafoods. The urinary excretion pattern of arsenic in man following oral ingestion of TMA contained in fishes once only indicates that the most portion of the TMA is excreted in urine (Tam et al. 1982; Luten et al. 1982; Yamauchi and Yamamura 1984). These experiments in humans have used fish arsenic but no authentic arsenobetaine. From their experiments in mice, rats and rabbits using ^{73}As-labeled arsenobetaine, Vahter et al. (1983) reported that arsenobetaine is not converted *in vivo* into any other chemical species of arsenic. From our previous studies in hamsters, we have attained the basic data on the metabolism, *in vivo* accumulation and excretion of arsenic trioxide (Yamauchi and Yamamura 1985a), methylarsonic acid (MAA) (Yamauchi and Yamamura 1985b) and dimethylarsinic acid (DMAA) (Yamauchi and Yamamura 1984a). The metabolic and excretory patterns of arsenic compounds in the hamster seem to be similar to those in humans (Yamauchi and Yamamura 1979; Buchet et al. 1981), and we believe that experiments of arsenobetaine for metabolic and excretory patterns in the hamster are of value.

In the present study, we examined arsenobetaine-treated hamsters for what chemical species of arsenic this arsenic compound (arsenobetaine) would be metabolized into *in vivo* and also for its excretory patterns in urine and feces with time.

MATERIALS AND METHODS

The authentic sample of arsenobetaine was synthesized according to the method of Edmond et al. (1977). It was identified by thin-layer chromatography and also directly by use of a gas chromatograph/mass spectrometer (JEOL model JMS-DX300) equipped with an FAB (fast atom bombardment) gun.

Male Syrian golden hamsters, weighing 92.7 ± 5.8 g, were used. The animals had free access to a pellet feed manufactured by Japan CLEA, Tokyo, and distilled water. They were administered orally with 35.6 mg/kg body weight of arsenobetaine, and killed before, and 1, 6, 12, 24, 72 and 120 hr after the administration. Urine and

feces were collected during the first 12 hr after the administration, and then every 24 hr by housing the hamsters in individual plastic metabolic cages. All materials were preserved frozen at -20°C until they were assayed. The hair was washed with distilled water, ethanol and acetone. In the assay for arsenic, 0.5 to 1 g of tissue or feces and 0.5 to 1 ml of blood or urine were used. The assay samples were transferred into 10-ml PMP (polymethylpentene) test tubes, and after the addition of 5 ml of 2N NaOH, heated in a heating block (YAMATO model HF-41) at 95°C for 3 hr. In preliminary experiments, we observed that neither MAA, DMAA nor arsenobetaine was degraded into any other species of arsenic even when heated at 95°C in 2N NaOH. Inorganic arsenic, MAA, DMAA and TMA were determined by atomic absorption spectrophotometry (Yamauchi and Yamamura 1984a). Arsenobetaine was determined as TMA. The detection limits of the 4 chemical species of arsenic by this method were 0.5 ng, the coefficient of variation being not less than 5%, respectively. From the amounts of inorganic arsenic, MAA, DMAA and TMA in urine and feces after the administration of arsenobetaine, the respective background values as described in the ***Results*** (mean values before the administration) were deducted.

RESULTS AND DISCUSSION

TMA concentrations in organs and tissues reached peaks at 1 and 6 hr after the oral administration of arsenobetaine once only, which occurred in the liver, kidney, lung, spleen, skin, muscle and brain in decreasing sequence of concentration (Table 1). No TMA was detected in the hair. TMA concentrations in the liver and kidney were especially high at 1 hr after the administration of arsenobetaine, and this deposition pattern had not been observed after the oral administration to hamsters of arsenic trioxide (Yamauchi and Yamamura 1985a), MAA (Yamauchi and Yamamura 1985b) or DMAA (Yamauchi and Yamamura 1984a) once only. This pattern may be interpreted to suggest that arsenobetaine is absorbed rapidly through the digestive tract, compared with arsenic trioxide, MAA and DMAA, and that it is excreted rapidly. From their experiments in mice, Vahter et al. (1983) reported the occurrence of peak arsenic (arsenobetaine) concentrations in organs and tissues at 1 hr after its administration. On the other hand, TMA concentrations in organs and tissues decreased rapidly from 12 hr after the administration, and were only similar to those in the control group or no more than traces at 120 hr after the administration. It was revealed that arsenobetaine disappears rapidly from organs and tissues and that it is an arsenic compound to be less likely retained *in vivo*. The arsenic detected in the liver at 1~120 hr after the administration of arsenobetaine was composed only of inorganic arsenic and TMA. The finding that inorganic arsenic concentrations in organs and tissues remained similar to those in the control group and that neither MAA nor DMAA was detected in organs and tissues, suggested that arsenobetaine is not converted *in vivo* into other chemical species of arsenic. Vahter et al. (1983) also reported similar findings. In other words, because Vahter et al. (1983) attained similar results by different analytical methods, we may conclude that arsenobetaine is not demethylated or is less likely demethylated *in vivo*.

Fig. 1 depicts changes in total arsenic concentration in whole blood following the oral administration of arsenobetaine once only. It reached a peak at 1 hr after the administration, when neither MAA nor DMAA but only inorganic arsenic and TMA were detected in blood. Inorganic arsenic concentration in blood remained similar to

Table 1. Arsenic concentrations in organs and tissues following oral administration of arsenobetaine once only

Materials	Chemical species	Arsenic concentration (μg As/g wet weight; hr after administration)						
		Control	1	6	12	24	72	120
Brain	Inorganic As	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	TMA	<0.01	0.23 ±0.08	0.21 ±0.10	0.07 ±0.02	0.02	<0.01	<0.01
Hair	Inorganic As	0.04 –	0.04 ±0.02	0.04 ±0.01	0.04 ±0.01	0.06 ±0.02	0.04 ±0.02	0.05 ±0.01
	TMA	–	–	–	–	–	–	–
Kidney	Inorganic As	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	TMA	<0.01	10.9 ±1.20	10.1 ±2.69	7.51 ±1.44	1.59 ±0.16	0.17 0.05	0.03
Liver	Inorganic As	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	TMA	0.01	32.7 ±2.78	14.7 ±5.34	3.08 ±0.50	0.58 ±0.30	0.08 ±0.04	0.04 ±0.01
Lung	Inorganic As	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	TMA	<0.01	5.98 ±2.16	6.32 ±1.49	2.27 ±0.29	0.62 ±0.29	0.06 ±0.02	0.02
Muscle	Inorganic As	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	TMA	<0.01	1.12 ±0.39	0.94 ±0.40	0.66 ±0.07	0.75 ±0.30	0.13 ±0.05	0.07 ±0.02
Skin	Inorganic As	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	TMA	–	1.84 ±0.72	1.37 ±0.42	0.63 ±0.10	0.21 ±0.07	0.04 ±0.02	0.03 ±0.01
Spleen	Inorganic As	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	TMA	0.02 –	4.33 ±1.91	5.20 ±1.90	2.04 ±1.21	0.76 ±0.14	0.16 ±0.05	0.03 ±0.01

Neither MAA nor DMAA was detected in organs and tissues.
–: not detected; Mean ± SD for 5 hamsters.

that in the control group even after the administration of arsenobetaine. TMA was found in high concentrations in plasma during the first 6 hr after the administration, and tended to occur in slightly higher concentrations in blood cells at 12 and 24 hr after the administration. Our previous experiments by oral administration of TMA to humans (Yamauchi and Yamamura 1984b) had also revealed a tendency to the occurrence of TMA in higher concentrations in plasma. From their experiments in mice and rabbits, Vahter et al. (1983) also reported similar findings.

Table 2 shows arsenic excretions in urine and feces following the administration of arsenobetaine. Deduction of the background value from the arsenic excretions in urine during the 120 hr after the administration led to the excretion of arsenic as only TMA in urine during the 120 hr after the administration. TMA excretion in urine was equivalent to 70.1% of the administered dose of arsenic by 12 hr after the

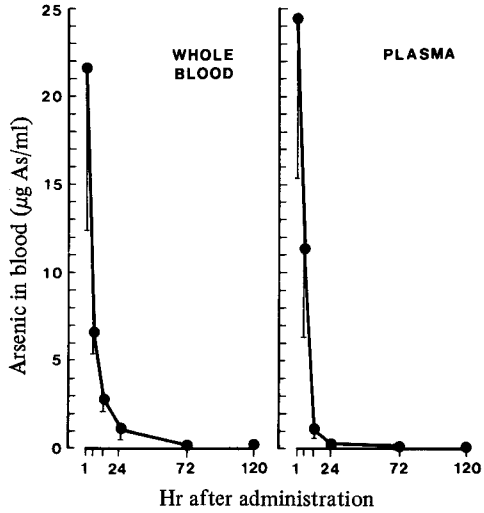

Figure 1. Arsenic concentration in blood after oral administration of arsenobetaine once only. Inorganic arsenic concentration in whole blood as well as in plasma was 0.03 μg As/ml. Neither MAA nor DMAA was detected.

Table 2. Urinary and fecal excretions of arsenic following oral administration of arsenobetaine once only.

Hr after administration	Concentrations of arsenic (μg As)									
	Inorganic arsenic		MAA		DMAA		TMA			
	Urine	Feces	Urine	Feces	Urine	Feces	Urine	%*	Feces	%*
~ 12	–	–	–	–	–	–	975 ±203	70.1	8.36±4.63	0.6
~ 24	–	–	–	–	–	–	195 ± 59.8	14.0	2.06±1.78	0.1
~ 48	–	–	–	–	–	–	46.4 ± 10.3	3.3	1.60±1.38	0.1
~ 72	–	–	–	–	–	–	28.7 ± 15.0	2.1	0.13±0.05	<0.1
~ 96	–	–	–	–	–	–	9.03± 2.53	0.6	0.20±0.08	<0.1
~120	–	–	–	–	–	–	5.06± 1.17	0.4	0.16±0.12	<0.1
1~120	–	–	–	–	–	–	1259 ±163	90.5	12.5 ±5.24	0.9

–: not detected; *: % ratio to dose (mean dose of 1391 μg As).
After correction for the basal daily excretion in urine (1.27±0.29 μgAs/day; 2% inorganic As, 2% MAA, 8% DMAA and 82% TMA) and in feces (0.31±0.03 μg As/day; 38% inorganic As, 6% MAA, 3% DMAA and 52% TMA).

administration, 84.1% by 24 hr after the administration, and 90.5% by 120 hr after the administration. From experiments in humans with fish arsenic (TMA or arsenobetaine), Tam et al. (1982) reported an excretion rate of 76.26%, Luten et al. (1982) reported a mean excretion rate of 75%, and Yamauchi and Yamamura

(1984b) reported 89.2 and 94.1%: all these authors described high urinary excretion rates of arsenic, and the finding in our present study supports the findings in these experiments in humans. Vahter et al. (1983) reproted that the half-life of i.v. arsenobetaine in the whole body of mice is no more than 12 hr. The finding in our present study that the urinary excretion of TMA during the first 12 hr after the administration was equivalent to about 70% of the administered dose of arsenic, suggests that the half-life of arsenobetaine may be not more than 12 hr: in other words, the finding by Vahter et al. (1983) agreed to our finding. Deduction of the background value from the excretions of arsenic in feces during the 120 hr after the administration led to the occurrence of arsenic as only TMA in feces. The TMA excretion in feces during the 120 hr after the administration was equivalent to only as small as about 1% of the administered dose of arsenic. In other words, it was shown that arsenobetaine is excreted chiefly into urine via the kidney.

When total arsenic excretion rates (in urine + feces) during the 120 hr after oral administration to hamsters of arsenic trioxide (Yamauchi and Yamamura 1985a), MAA (Yamauchi and Yamamura 1985b), DMAA (Yamauchi and Yamamura 1984a) and arsenobetaine once only are compared, the last is the highest, that is, about 91%, which is about 1.5 times as high as that following the administration of arsenic trioxide. It was also revealed that there was no significant difference in total arsenic excretion rate in hamsters between the administrations of arsenobetaine, MAA and DMAA. The excretion of arsenic compounds varies with chemical species and also with chemical structure, but it was postulated that this difference would occur only when arsenic compounds are classed simply into inorganic arsenic and methylarsenic compounds.

Arsenobetaine proved to be an arsenic compound which, when administered orally, is absorbed rapidly through the digestive tract and which is mostly excreted into urine in a short time, being less likely retained *in vivo*. In a previous paper (Yamauchi and Yamamura 1983), we described that TMA was less likely detected in human organs and tissues, and the finding in the present study in experimental animals may be thought to support the behavior of TMA in human organs and tissues. From its physicochemical properties, arsenobetaine does not seem to impair our health even though we ingest a large amount of this arsenic compound from seafoods.

Acknowledgements. We are grateful to Trichemical Co., Kanagawa, Japan, for cooperation in the synthesis of the sample of arsenobetaine used in the present study.

REFERENCES

Buchet JP, Lauwerys R, Roels H (1981) Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. Ind Arch Occup Environ Health 48:71–79

Cannon JR, Edmonds JS, Francesconi KA, Raston CL, Saunders JB, Skelton BW, White AH (1981) Isolation, crystal structure and synthesis of arsenobetaine, a constituent of the western rock lobster, the dusky shark, and some samples of human urine. Aust J Chem 34:787–798

Edmonds JS, Francesconi FA, Cannon JR, Raston CL, Skelton BW, White AH (1977) Isolation, crystal structure and synthesis of arsenobetaine, the arsenical constituent of the western rock lobster *Panulirus longipes cygnus* George. Tetrahedron Lett 18:1543–1546

Luten JB, Riekwel-Booy G, Rauchbaar A (1982) Occurrence of arsenic in plaice (*Pleuronectes platessa*), nature of organoarsenic compound present and its excretion by man. Environ Health Perspect 45:165–170

Tam GKH, Charbonneau SM, Bryce F, Sandi E (1982) Excretion of a single oral dose of fish-arsenic in man. Bull Environ Contam Toxicol 28:669–673

Vahter M, Marafante E, Dencker L (1983) Metabolism of arsenobetaine in mice, rats and rabbits. Sci. Total Environ 30:197–211

Yamauchi H and Yamamura Y (1979) Dynamic change of inorganic arsenic and methylarsenic compounds in human urine after oral intake as arsenic trioxide. Ind Health 17:79–83

Yamauchi H and Yamamura Y (1983) Concentration and chemical species of arsenic in human tissue. Bull Environ Contam Toxicol 31:267–270

Yamauchi H and Yamamura Y (1984a) Metabolism and excretion of orally administered dimethylarsinic acid in the hamster. Toxicol Appl Pharmacol 74:134–140

Yamauchi H and Yamamura Y (1984b) Metabolism and excretion of orally ingested trimethylarsenic in man. Bull Environ Contam Toxicol 32:682–687

Yamauchi H and Yamamura Y (1985a) Metabolism and excretion of orally administered arsenic trioxide in the hamster. Toxicology 34:113–121

Yamauchi H and Yamamura Y (1985b) Metabolism and excretion of orally administered methylarsonic acid in the hamster. Jpn J Ind Health 27:40–41

Received July 12, 1985; accepted August 12, 1985.