DYNAMIC CHANGE OF INORGANIC ARSENIC AND METHYLARSENIC COMPOUNDS IN HUMAN URINE AFTER ORAL INTAKE AS ARSENIC TRIOXIDE

Hiroshi YAMAUCHI and Yukio YAMAMURA

Department of Public Health, St. Marianna University, School of Medicine,
Sugao, Takatsu-ku, Kawasaki, 213 Japan

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The outputs of inorganic arsenic compound (As^{+3} , As^{+5}), monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) in the urine were observed with passage of time after oral intake of arsenic trioxide. The subject was completely restricted from the ingestion of seafoods for two days before the experiment and during the urine sampling period, because of high arsenic content of these foods. As^{+3} was excreted in large amounts during the first 12 hours, the outputs corresponding to about 12% of the ingested amount, and the outputs of As^{+3} also were greater than those of MMAA and DMAA during the same period. The urinary outputs of arsenic compounds as total arsenic following the ingestion of arsenic trioxide amounted to 40% of the ingested amount during the first 12 hours, and 70% by 72 hours. Under restricted ingestion of seafoods, if the ratios of arsenite to total arsenic increase in the urine of arsenic workers, it is of importance as suggesting an increased absorption of toxic arsenite.

It has been shown that inorgano-arsenic compounds in the environment are methylated by microorganisms into dimethylarsinic acid, and dimethylarsinic acid is that most ubiquitous chemical form of arsenic in the biologic system. Braman and Forebeck (1973)¹⁾ developed a method for the isolation, separation and assay of the inorgano-arsenic compounds, i.e., arsenite (As^{+3}) and arsenate (As^{+5}) and also the organo-arsenic compounds, i.e., monomethylarsonic acid (MMAA) and demethylarsinic acid (DMAA), and as a result of assay of human urine for arsenic, reported that DMAA accounted for 40–90% of the arsenic compounds in the urine. Crecelius (1977)²⁾ also described that DMAA was excreted in the urine at the highest concentration after ingestion of As^{+3} -rich table wine, As^{+5} -rich well water, or organo-arsenic-rich Dungeness crab (*Cancer magister*).

In a previous paper (1979)³⁾, we described the urinary output patterns of arsenic compounds following ingestion of an arsenate-rich seaweed extract. The purpose of this study is to indicate the dynamic change of inorganic arsenic and methylarsenic compounds in human urine after oral intake as arsenic trioxide.

METHODS

- (1) As^{+3} solution ingested: 0.132g As_2O_3 (Merck AG: 99.9% purity) was dissolved in 10% solution of sodium bicarbonate, and the solution was diluted with distilled water.
- (2) The subject was a 28-year-old male weighing 70 kg, who ingested 700 μg of As^{+3} as the above-mentioned solution. Ingestion of marine products was completely restricted for 2 days before each experiment and also during the urine sampling period. The urine was collected during the period from 24 hours before the ingestion of the As_2O_3 solution until 72 hours after the ingestion.
- (3) Separation and assay of urine As^{+3} , As^{+5} , MMAA and DMAA in the urine: To each urine sample were added 10% oxalate solution and 10% phthalate solution as buffers. Arsenic contained in the mixture was reduced into arsines with 10% sodium borohydride solution, and the arsines were then fixed with liquid nitrogen. From the fixed arsines was separated the component arsines, which were determined by arsine generation-flameless atomic absorption spectrophotometry.³⁾

RESULTS AND DISCUSSION

Fig. 1 shows changes in urinary As^{+3} , As^{+5} , MMAA and DMAA outputs following the ingestion of As^{+3} . Ingestion of fishes and shellfishes had been restricted for 2 days before each experiments, and a 24-hour urine was assayed for the normal background

Fig. 1. Amounts of arsenic compounds in urine with time after oral intake of arsenic trioxide (10 μg As/Kg).

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levels of the 4 chemical forms of arsenic. The subject then ingested 700 μg of As^{+3} , and the whole urine samples collected at 1, 2, 4, 6, 8, 10 and 12 hours and then at 6-hours intervals were assayed for the outputs of the 4 chemical forms of arsenic. The top half of Fig. 1 shows the results of the 1st experiment, and the bottom half, those of the 2nd experiment. Table 1 shows the normal background levels of the 4 chemical

Table 1. The normal background levels of arsenic compounds in urine after restriction of seafood ingestion.

As^{+3}	As^{+5}	MMAA	DMAA	Total-As
0.24	0.08	0.62	1.21	2.15
± 0.07	± 0.07	± 0.18	± 0.41	± 0.70

Mean $\pm$ S.D. of 16 samples
 $\mu\text{g As/hr.}$

forms of urinary arsenic. Table 2 gives the findings by following up the 4 chemical forms of arsenic in the urine samples collected at the aforementioned intervals for 72 hours following the oral ingestion of 700 μg of As^{+3} , which were obtained by deducting the background levels shown in Table 1 from the determined outputs.

As^{+3} was excreted in large amounts within the first 12 hours, and the amount of this output corresponded to about 12% of the ingested amount. Also, of the total output of As^{+3} during the 72-hour period, 1/3 was excreted during the first 12 hours. Furthermore, the urinary As^{+3} level was as low as the background level at 72 hours. The urinary outputs of MMAA and DMAA were at their peaks 6-12 hours after the ingestion of As^{+3} , and remained at the high level until about 48 hours after the ingestion. Especially, both DMAA and MMAA remained at higher levels than As^{+3} at 12 hours and all the subsequent stages. As^{+5} increased considerably in the urine within 12 hours despite the ingestion of As^{+3} . This increased As^{+5} output returned to the background level at 60 hours.

Crecelius²⁾ observed four chemical forms of arsenic excreted in the urine following ingestion of As^{+3} -rich table wine (containing 50 μg of As^{+3} and 13 μg of As^{+5}), and described that 80% of the ingested amount of arsenic was excreted in the urine within 61 hours, and that, when the excreted arsenic compounds were analyzed for their chemical forms, DMAA made up 50%; As^{+3} , 8%; As^{+5} , 8%; and MMAA, 14% of the arsenic output. The present study differed from that by Crecelius where As^{+3} itself was ingested in the former, and the characteristic of this study was the output of As^{+3} increased markedly immediately after the ingestion.

In the next place, in order to investigate for whether there is a difference of the velocity between the urinary excretion between As^{+3} and As^{+5} , the results of this study were compared with those of the previous study³⁾ in which an As^{+5} -rich sample was ingested (out of the subjects selected for the previous study,³⁾ subject B was the same

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Table 2. Urinary arsenic compounds excretion following oral intake of arsenic trioxide (10 μg As/Kg).

Experiment No.	Time after ingestion	Amounts of arsenics speies in urine									
		As (III)		As (V)		MMAA		DMAA		Total-As	
		μg	%†	μg	%†	μg	%†	μg	%†	μg	%†
1st	0-12	68.5	9.8	21.0	3.0	34.1	4.9	32.9	4.7	156.5	22.4
2nd		95.1	13.6	29.0	4.1	47.6	6.8	45.4	6.5	217.1	31.0
$\bar{x}$		81.8	11.7	25.0	3.6	40.9	5.9	39.2	5.6	186.8	26.7
1st	12-24	22.9	3.3	5.8	0.8	31.0	4.4	38.5	5.5	98.2	14.0
2nd		21.0	3.0	5.0	0.7	35.2	5.0	33.1	4.7	94.3	13.5
$\bar{x}$		22.0	3.2	5.4	0.8	33.1	4.7	35.8	5.1	96.3	13.8
1st	24-36	24.7	3.5	4.6	0.7	30.7	4.4	46.5	6.6	106.5	15.2
2nd		15.8	2.3	4.9	0.7	21.3	3.0	28.9	4.1	71.0	10.1
$\bar{x}$		20.3	2.9	4.8	0.7	26.0	3.7	37.7	5.4	88.8	12.7
1st	36-48	18.5	2.6	1.7	0.2	29.7	4.2	34.9	5.0	84.8	12.1
2nd		8.8	1.3	2.0	0.3	14.4	2.1	14.6	2.1	42.7	6.1
$\bar{x}$		13.7	2.0	1.9	0.3	22.1	3.2	24.8	3.6	63.8	9.1
1st	48-60	10.1	1.4	0.9	0.1	7.3	1.0	13.8	2.0	32.1	4.6
2nd		6.0	0.9	0.6	0.1	7.5	1.1	6.7	1.0	22.2	3.2
$\bar{x}$		8.1	1.2	0.8	0.1	7.4	1.1	10.3	1.5	27.2	3.9
1st	60-72	5.2	0.7	0	0	2.6	0.4	3.6	0.5	11.4	1.6
2nd		5.4	0.8	0	0	7.6	1.1	8.8	1.3	22.4	3.2
$\bar{x}$		5.3	0.8	0	0	5.1	0.8	6.2	0.9	16.9	2.4
Total-As											
1st		152.1	21.7	34.0	4.9	133.6	19.1	137.5	19.6	469.7	67.1
2nd		149.9	21.4	41.5	5.9	135.4	19.3	170.2	24.3	489.5	69.9
$\bar{x}$		151.0	21.6	37.8	5.4	134.5	19.2	153.9	22.0	479.6	68.2

† % of dose (700 μg As), MMAA; Monomethylarsonic acid, DMAA; Dimethylarsinic acid

subject as in this study). The total urinary output of arsenic during the 48 hours after the ingestion of As^{+5} was 39% of the ingested amount,³⁾ while the total urinary output of arsenic [during the first 48 hours after its ingestion in this study was 62% of the ingested amount.

This is rather contrary to the result reported by Schroeder et al⁴⁾ that As^{+5} was excreted more rapidly than As^{+3} .

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CONCLUSION

The urinary outputs of As^{+3} , As^{+5} , MMAA and DMAA were observed during the period from 1 to 72 hours after oral ingestion of As_2O_3 (As^{+3}) only once.

- (1) The urinary outputs of arsenic compounds as total arsenic following the ingestion of As^{+3} amounted to 40% of the ingested amount within the first 12 hours, and 70% within 72 hours.
- (2) When the urinary output of arsenic compounds during the 72 hours after the ingestion of As^{+3} was analyzed by the chemical forms of arsenic, As^{+3} accounted for 22%; As^{+5} , for 5%; MMAA, for 19%, and DMAA, 22% of the output.
- (3) The output of As^{+3} was greater than that of MMAA and of DMAA within the first 12 hours, and corresponded to about 12% of the ingested amount of As^{+3} .
- (4) Both DMAA and MMAA in urine remained at higher levels than As^{+3} at all stages 12 hours or more after the ingestion of As^{+3} , and still remained at slightly higher levels than the normal background levels even at 72 hours.
- (5) Only traces of As^{+5} were detected, which appeared suggestive of oxidation of part of As^{+3} into As^{+5} in vivo.

The ingestion of As^{+3} resulted in markedly increased urinary outputs of As^{+3} for the following several hours. From this result, if the high ratios of arsenite to total arsenic are found in the urine of arsenic workers under restricted ingestion of seafoods, it is of importance as suggesting an increased absorption of toxic arsenite.

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