Occurrence of Monomethylarsonous Acid in Urine of Humans Exposed to Inorganic Arsenic

ARTICLE *in* CHEMICAL RESEARCH IN TOXICOLOGY · SEPTEMBER 2000

Impact Factor: 3.53 · DOI: 10.1021/tx000114o · Source: PubMed

Communications

Occurrence of Monomethylarsonous Acid in Urine of Humans Exposed to Inorganic Arsenic

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Received May 24, 2000

Monomethylarsonous acid (MMA^{III}) has been detected for the first time in the urine of some humans exposed to inorganic arsenic in their drinking water. Our experiments have dealt with subjects in Romania who have been exposed to 2.8, 29, 84, or 161 μg of As/L in their drinking water. In the latter two groups, MMA^{III} was 11 and 7% of the urinary arsenic while the monomethylarsonic acid (MMA^{V}) was 14 and 13%, respectively. Of our 58 subjects, 17% had MMA^{III} in their urine. MMA^{III} was not found in urine of any members of the group with the lowest level of As exposure. If the lowest-level As exposure group is excluded, 23% of our subjects had MMA^{III} in their urine. Our results indicate that (a) future studies concerning urinary arsenic profiles of arsenic-exposed humans must determine MMA^{III} concentrations, (b) previous studies of urinary profiles dealing with humans exposed to arsenic need to be re-examined and re-evaluated, and (c) since MMA^{III} is more toxic than inorganic arsenite, a re-examination is needed of the two hypotheses which hold that methylation is a detoxication process for inorganic arsenite and that inorganic arsenite is the major cause of the toxicity and carcinogenicity of inorganic arsenic.

Introduction

There have been many investigations of the arsenic species in human urine (for reviews, see refs *1*−*3*). These have resulted in reports expressing the arsenic species as a percentage of the total arsenic in the urine, for example, % inorganic arsenic (inorg As),[1] % monomethylarsonic acid (MMA), and % dimethylarsinic acid (DMA). In such studies, the MMA has been believed to be monomethylarsonic acid (MMA^{V}) (*1*−*3*). The studies, however, did not measure the level of urinary monomethylarsonous acid (MMA^{III}) (Figure 1).

Our laboratory has studied intensively the enzymology of the biotransformation of inorganic arsenic (*4*−*12*) and has demonstrated that MMA^{V} reductase, which catalyzes the synthesis of MMA^{III}, is the rate-limiting enzyme (*11*). In 1989, Cullen et al. (*13*) proposed MMA^{III} as an intermediate in the conversion of inorg As to DMA.

In one of our previous studies, in which the chelating agent DMPS was given to native Chileans chronically exposed to inorganic arsenic in their drinking water, an unknown peak was found on an HPLC chromatogram during the analyses of their urine (*14*). Subsequently, after DMPS was given to subjects in Inner Mongolia, MMA^{III} was identified in the urine of some subjects exposed to inorg As (*15*). But for those subjects, however, the MMA^{III} was found in the urine only after DMPS

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[1] Abbreviations: MMA, generic term that includes MMA^{III} and MMA^{V}; MMA^{III}, monomethylarsonous acid; MMA^{V}, monomethylarsonic acid; DMA, dimethylarsinic acid; inorg As, inorganic arsenic; DMPS, sodium 2,3-dimercapto-1-propanesulfonate.

10.1021/tx000114o CCC: $19.00
Published on Web 07/08/2000

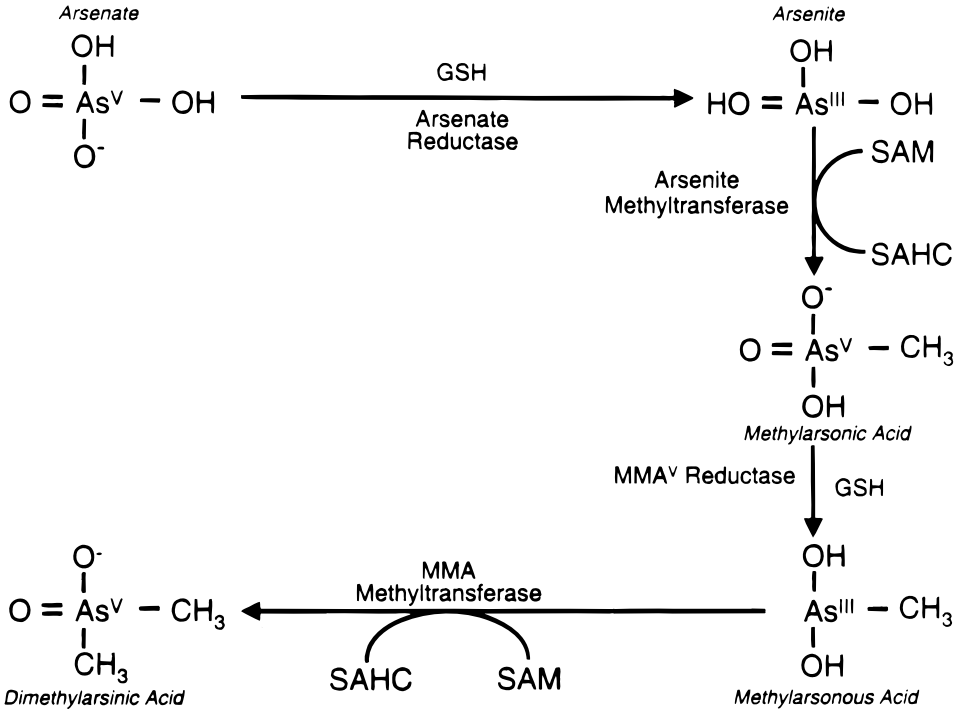

Figure 1. Pathway for biotransformation of inorganic arsenic. SAM is *S*-adenosylmethionine and SAHC *S*-adenosylhomocysteine.

Table 1. Demographic Factors Associated with Subjects

locality	Chrisineu Cris	Zerind	Sepreus	Ciumeghiu
population	10000	1600	2757	2056
no. of subjects	14	14	14	16
no. of women	7	7	7	8
no. of men	7	7	7	8
drinking water				
our analysis				
As^V (μg/L ± SD)	2.8[a]	23.5 ± 0.1[b]	29.1 ± 0.7	87 ± 2[c]
As^{III} (μg/L ± SD)	nd	5.0 ± 0.3[b]	55 ± 2	74 ± 1[c]
sum	2.8	29	84	161
government data				
As (μg/L)	6	42	99	171
depth of well (m)	100	370	150	200
year established	1976	1904	1949	before 1966

[a] Drinking water in this area is obtained from a central water facility dug in 1976 that is 100 m deep. [b] This value is for water from the old well of this village. Of the 14 subjects in this group, all drank from this old well, but four also drank water from a new well dug in 1986 which is 100 m deep; the government analysis of this well was 58 μg of As/L. [c] By our analysis, the new well contained As^V (46.8 ± 0.7 μg/L), and As^{III} could not be detected.

administration, raising the question of whether it was an endogenously formed metabolite or whether DMPS in the urine reduced the MMA^V to MMA^{III}. With the development of a new, very sensitive and reliable method for analysis of arsenic species in urine crafted by Le et al. (*16*), many questions became answerable.

This paper reports the presence or absence of MMA^{III} in the urine of Romanian subjects drinking water containing different amounts of inorg As. These subjects had not previously received DMPS or any other metal-complexing agent as had the Chilean and Inner Mongolian subjects in earlier studies.

Millions of people are at risk of hyperpigmentation and skin cancer due to drinking water that contains excessive amounts of inorg As in Chile (*17*), Bangladesh (*18*), India (*19*, *20*), Taiwan (*21*), Romania (*22*), China (*23*), and Mexico (*24*). The results of this study strongly indicate a new, perhaps selective, biomarker for arsenic exposure that warrants extensive study in helping to decipher the enigma of arsenic carcinogenesis in humans.

Experimental Procedures

The subjects of this study were all in good health. The villages and towns where the subjects lived were in proximity to the city of Arad in Arad County, Romania. The subjects were transported by bus or van from their homes to a motel in the village of Nadab where they spent the night and urine samples were collected. The motel was approximately 5 km from Chrisineu Cris, 20 km from Zerind, 22 km from Sepreus, and 30 km from Ciumeghiu, the towns and villages in which the subjects lived (Table 1).

Our team collected water from each of the wells used by the subjects. Water was analyzed by the same method used to analyze the urine (*16*). The water temperature was not measured at the time of collection, which might account for the different amounts of arsenate and arsenite in the water from different wells (Table 1). Our measurement of arsenic concentration (Table 1) differed from the government results. This difference may be the result of timing and analytical methodology.

Protocol. Subjects provided a medical history and underwent a physical examination before being enrolled in the study. The examining physicians were L. Yip and A. Gurzau. Our experi-

Table 2. MMA^{III} in Human Urine

group	water (μg of As/L)	no. in group	no. of subjects having MMA^{III} in urine	MMA^{III} in urine[a] (μg/11 h ± SE)	MMA^{III} (μg/L ± SE)
A	2.8	14	0[b]	nd[f]	nd
B	28.5	14	1[c]	12.0	4.8
C	85.5	14	4[d]	4.5 ± 1.5	5.7 ± 2.2
D	161.2	16	5[e]	5.1 ± 1.6	6.9 ± 2.6

[a] The values in the last two columns are the means only of those urines in which MMA^{III} was detected. [b] No subject in this group had detectable amounts of MMA^{III} in the urine. [c] One man. [d] One man and three women. [e] Four men and one woman. [f] nd, not detectable.

mental protocol was approved by the Human Subjects Committee of The University of Arizona. Participants were asked to exclude seafood from their diet for the preceding 3 days. Each participant was familiarized with the protocol and signed a consent form. Adults who were 18−69 years of age were included in this study. Prior information was available with respect to the arsenic content of the drinking water of each subject. Excluded were subjects with a history of current physical findings of serious renal or psychiatric disease, subjects with abnormalities in blood tests or urinalysis that in the physican's opinion would interfere with the study, subjects who had received any investigational drug during the preceding month before the initiation of this study, subjects who had taken drugs with well-defined organ toxicity within the past 6 months, subjects who were pregnant or lactating, subjects with a history of alcohol or recreational drug abuse, and subjects who were not capable of giving informed consent.

Subjects fasted for 11 h. Urine collected for 11 h overnight (9 p.m. to 8 a.m.) during which time subjects were allowed to drink distilled water, ad libitum.

Urine Collection. The procedures for cleaning collecting plasticware and for urine collection were identical to those previously described (*14*, *15*). Urine samples were immediately frozen by placing them in a freezer at the study site. The samples were kept frozen while being transported by air to Tucson, where they were stored at −20 °C for approximately 3 months.

Arsenic Analysis. Samples were shipped by air to Edmonton, AB, for analysis. Analyses were carried out (X. Chris Le, University of Alberta) using HPLC-hydride generation/atomic fluorescence spectrometry (*16*, *25*). The methods used to analyze for arsenic species, detection limits, quality control, precision, and sensitivity of these analytical measurements and validation of the procedures have been described in detail by Le et al. (*16*). The values that were determined for arsenic species of individual urines were mean values from triplicate analyses. The detection limits were as follows: As^{III} and MMA^{V}, 0.5 μg/L; As^{V} and DMA^{V}, 1 μg/L; and MMA^{III}, 2 μg/L. The analyst did not know the identity of the samples or the village from which they were obtained.

Results

Although the number of subjects (14−16) in each group was small, a dose−response relationship for As in drinking water and urinary MMA^{III} was found. When exposure occurred via drinking water containing 2.8 μg of As/L, no urinary MMA^{III} was detected in the urine of the subjects (Table 2). At 29 μg of As/L, 7% of the group, at 84 μg of As/L, 29% of the group, and at 161 μg of As/L 31% of the group had MMA^{III} in their urine. No conclusions can be reached from the results with low water As concentrations. However, the groups with higher concentrations exhibited higher levels of urinary MMA^{III} (Table 2).

The appearance of MMA^{III} in the urine does not appear to be related to gender. Only one man drinking from the 29 μg of As/L well had MMA^{III} in his urine. Of the 84 μg of As/L group one man and three women and of the 161 μg of As/L group four men and one woman had urinary MMA^{III}. Further studies with a greater number of subjects is needed before any gender-related conclusions can be made. Percentage data for MMA^{III} are reported in Table S1 in the Supporting Information.

Percent of Arsenic Species. Previously, urinary arsenic species data have been converted to the percentage of the total urinary arsenic (*1*−*3*). In the absence of MMA^{III} percentage determinations, MMA% would have been estimated to be 25 and 20 for groups C and D, respectively. With the determination of individual MMA^{V} and MMA^{III} percentages, 13−14 and 7−11%, respectively (Table S1), we can calculate that 44% (group C) and 35% (group D) of total MMA is MMA^{III}, depending on the arsenic content in the water.

Discussion

We have found MMA^{III} in the urine of some humans exposed to inorg As in their drinking water. Our previous observations (*26*) and those of Cullen et al. (*13*) and Styblo et al. (*27*) that MMA^{III} is more toxic than inorganic arsenite indicate that a critical re-examination of the many hypotheses dealing with arsenic toxicity is necessary. For example, methylation may not be a detoxication process for inorganic arsenite. Arsenite, per se, may not be the major cause of the published toxicity and carcinogenicity of inorg As.

In addition, this work forces a number of major questions to be answered. First of all, why did only 10 of the 58 subjects (17%) have measurable amounts of MMA^{III} in their urine? The percentage is higher, 10 of 44 (23%), if the groups having at least one member with MMA^{III} in their urine are considered. Are the subjects who excreted MMA^{III} part of a more susceptible population whose members process inorg As or MMA^{III} in a different manner or at a very different rate? For example, if MMA^{III} is normally bound to specific thiol, dithiol, or protein sites, do the members of this subpopulation have fewer such sites? If so, have such sites become occupied due to the higher arsenic intake, resulting in MMA^{III} spilling out in the urine? There has been a great deal of variation in the amount of arsenic species excreted by humans (*28*). Are these variations in any way related to MMA^{III}?

Second, does the concentration of urinary arsenic species depend on the intracellular MMA^{V} concentration? MMA^{V} reductase is the rate-limiting enzyme of the inorganic arsenite biotransformation pathway (*11*). It is pertinent to note that experiments with partially purified MMA^{V} reductase showed that the concentration of MMA^{V} required for the reaction to reach half-maximum velocity is in the millimolar concentration range, whereas that for the other enzyme reactions of the inorganic arsenite biotransformation pathway is in the micromolar range (*11*). For significant amounts of MMA^{V} to be converted to the more toxic MMA^{III}, the MMA^{V} concentration must reach the millimolar range. Otherwise, most of the MMA^{V} will be excreted in the urine or become bound to tissue. Thus, it would appear that the percentage of each of the arsenic species found in the urine may depend on the

[2] W. R. Cullen, personal communication.
[3] Unpublished results.

concentration of MMA^V in the cell, the rate of urinary excretion of MMA^V, the rate of MMA^V reductase, and the rate of MMA^{III} methyltransferase. At lower concentrations, MMA^V can be expected to be tissue-bound and/or excreted. When the MMA^V concentration in the cell reaches a critical millimolar level, more of it will be converted to the more toxic MMA^{III}. When arsenic exposure increases and the MMA^V concentration increases, MMA^{III} begins to form. Some MMA^{III} may accumulate in tissues, leading to toxicity; some may be excreted; and some is converted to DMA^V. It is also possible that some MMA^{III} or DMA^V is demethylated. MMA and DMA can be demethylated by some bacteria and fungi.[2]

Another source of high levels of MMA^{III} in the urine may be polymorphic, less active MMA^{III} methyltransferase. Indisputable molecular biology evidence, however, has not been found for polymorphism in the biotransformation of arsenic; however, we have found two major peaks of arsenic methyltransferase activity while purifying on DEAE-cellulose and other ion exchange columns.[3] The two peaks of enzyme activity have similar substrates and other properties.

The implications of our finding MMA^{III} in the urine of some humans exposed to arsenic should be apparent due to the greater toxicity of MMA^{III} (*26*, *27*) as well as the genotoxic and carcinogenic potential of DMA (*29*, *30*). In addition, we have recently detected MMA^{III} and DMA^{III} in the livers of hamsters given radioactive arsenate. Since previous methods used to analyze urine and tissues did not differentiate between MMA^V and MMA^{III}, published data need to be re-examined and perhaps reinterpreted. In addition, the question of whether there is a correlation between urinary MMA^{III} concentrations and arsenic-induced hyperpigmentation and/or skin cancer needs to be answered.

Our studies have dealt with humans who have been exposed to lower amounts of arsenic than those that are now of worldwide concern. Experiments are now being planned to determine whether a correlation exists between the urinary MMA^{III} level and the signs of greater arsenic exposure such as hyperpigmentation and skin cancer.

Acknowledgment. This work was supported in part by Superfund Basic Research Program NIEHS Grant ES-04940 from the National Institute of Environmental Health Sciences and Southwest Environmental Health Sciences Center Grant P30-ES-06694.

Supporting Information Available: Table S1 reporting concentration levels of MMA^{III}. This material is available free of charge via the Internet at http://pubs.acs.org.

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