APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 10, 741–745 (1996)

Methylation and Demethylation of Dimethylarsinic Acid in Rats Following Chronic Oral Exposure

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Metabolites of dimethylarsinic acid (DMA) were studied in rats chronically exposed to DMA in drinking water. The urine was collected by forced urination at the end of 8, 20 and 30 weeks and the feces at the end of 30 weeks. The samples were analyzed for arsenic species by a combined system of ion chromatography and inductively coupled plasma mass spectrometry (IC–ICP–MS). Increases in arsenite, DMA, trimethylarsine oxide and a still-to-be-identified arsenic compound (which was eluted immediately after monomethylarsonic acid on the chromatogram) were detected in both urine and feces. At the 100 mg l^{-1} dose, DMA was the main component in the urine; arsenite was a main component in the feces. The results indicate that, besides undergoing methylation, DMA can be demethylated to inorganic arsenic, and demethylation of DMA may be associated with intestinal bacteria.

Keywords: dimethylarsinic acid; chronic exposure; demethylation; arsenite; rat; urine; feces; methylation; inductively coupled plasma mass spectrometry (ICP–MS); ion chromatography (IC)

INTRODUCTION

Arsenic is a ubiquitous trace element found in various compounds which are widely distributed in the environment,[1] and there have been reports of various mechanisms of exposure for humans.[2] Epidemiological studies have shown that in humans arsenic is carcinogenic to the skin and lungs, and that cancer incidence is associated with the level and exposure period of arsenic.[3–5] It is well known that the biological availability and the toxicological effects of arsenic depend on the chemical forms. Most mammals, including human beings, are able to methylate inorganic arsenic compounds to methylated arsenics. Dimethylarsinic acid(DMA) is the major methylated metabolite of inorganic arsenic,[6,7] and can be further methylated to trimethylarsine oxide (TMAO).[8,9] However, in previous studies, there were no indications that DMA could be demethylated to inorganic arsenic *in vivo*.[10,11] Methylation can be considered to be a detoxication, because methylated arsenic compounds have lower toxicity and lower affinity for tissue constituents, and they can be eliminated more quickly than inorganic arsenic.[12] On the other hand it has been reported that, with chronic exposure, DMA may act as a carcinogen or a promoter in rats, and that tumor inductions in kidney and thyroid glands were moderately enhanced by DMA in a dose-dependent manner.[13,14] DMA induces tetraploids and mitotic arrest in Chinese hamster cells,[15] and causes DNA damage in mice.[16] A few studies on the metabolism of DMA have been conducted but there have been no reports concerning chronic exposure to DMA. On the other hand, the effects of arsenic on humans are often due to chronic exposure. The mechanism of DMA toxicity has not yet been resolved.

In this study, DMA was administered to rats in drinking water for 30 weeks, and the metabolites of DMA in the urine and the feces were determined by a combined system of ion chromatography (IC) and inductively coupled plasma mass spectrometry (ICP–MS). At the same time,

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CCC 0268–2605/96/090741–05

Received 5 December 1995
Accepted 12 January 1996

the effects of DMA on chemical carcinogenesis was studied by co-workers. Inoue's group[17,18] reported that the IC–ICP–MS system is a sensitive, accurate and precise analytical method for arsenic compounds in the urine of DMA-exposed rats. The IC–ICP–MS system not only demonstrates low detection limits, but also it does not require sample preparation. Therefore, any artificial products generated in the sample preparation process can be eliminated.

MATERIALS AND METHODS

Animals

F344/Du Crj male rats were obtained at 5 weeks of age from Charles River Japan Inc. (Hino, Japan). Rats were housed at four per steel cage, with wood-chip bedding and were fed with common basal diet (CE2, Clea Japan Inc., Tokyo, Japan) and given water *ad libitum*. The rats were kept at 23±1 °C with a 12/12-h light/dark cycle. After a one-week acclimation period, they were used in this study.

Chemicals

DMA for administration was purchased from Wako Pure Chemical Industries (Osaka, Japan). Its purity was 99%, and inorganic arsenic was not detected in samples subjected to IC–ICP–MS. Sodium arsenite, monomethylarsonic acid (MMA), DMA, trimethylarsine oxide (TMAO) and arsenobetaine (AsBe), with purities of 99.99%, were purchased from Trichemical Lab. (Yamanashi, Japan) and used as standard solutions for IC–ICP–MS determinations.

Experimental procedure

In two groups, each consisting of 12 rats, 0 and 100 mg l^{-1} DMA were given in drinking water for 30 weeks beginning at 10 weeks of age. In another six groups, each consisting of 20 rats, the carcinogenic initiator, 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN), was given in drinking water from 6 weeks to 10 weeks of age; then 0, 2, 10, 25, 50 and 100 mg l^{-1} DMA respectively were administered for 30 weeks in the drinking water. The urine was collected by forced urination at the end of 0, 8, 20 and 30 weeks, and the feces were collected by forced defecation at the end of 30 weeks. The samples were stored at −20 °C until analyzed.

Determination of arsenic speciation in urine and feces

A combined system with IC as the separation technique (model IC 7000, Yokogawa Analytical Systems Inc., Tokyo, Japan) and ICP–MS (model HP4500, Hewlett-Packard Co., DE, USA) as an element-selective detector was used to analyze arsenic species. For separation of the five arsenite compounds, arsenite, MMA, DMA, TMAO and AsBe, the IC separation column was the Excelpack ICS-A35 (150 mm×4.6 mm i.d.) packed with a polymer-based hydrophilic anion-exchange resin (Yokogawa Analytical Systems Inc., Tokyo, Japan). IC was operated under the following conditions: mobile phase, 10 mM tartaric acid; flow rate, 1.0 ml min^{-1}; column temperature, 50 °C; and injection volume, 50 µl. The operational conditions for the ICP–MS were given as follows: radio-frequency forward power, 1300 W; radio-frequency reflected power, <1 W; plasma gas flow, 16.0 l min^{-1}; auxiliary gas flow, 1.00 l min^{-1}; carrier gas flow, 1.06 l min^{-1}; sampling point, 6 mm from load coil; dwell time, 0.5 s. The eluent from the ion-chromatography column was introduced directly into the nebulizer of the ICP–MS and analyzed at monitoring mass of 75.

Arsenic compounds were determined by a modification of the method of Inoue and co-workers.[17,18] Stock standard solutions of sodium arsenite, MMA, DMA, TMAO and AsBe were prepared by dissolving each arsenic compound in pure water at a concentration of 100 mg As l^{-1}. The final dilute aqueous standard mixtures were prepared from each stock standard just before use. The urine samples were diluted 20-fold with pure water and 50 µl of the diluted urine was injected into the IC–ICP–MS system. The feces were homogenized by an Ultra-Turrax homogenizer (Cosmo Bio Inc., Tokyo, Japan) in 10 mM tartaric acid. The homogenized suspension was centrifuged and the supernatant was filtered through a 0.45 µm syringe filter (Whatman Lab, NJ, USA). Then 50 µl of the filtrate was injected into the IC–ICP–MS for analysis.

RESULTS

At the end of three weeks, body weight gains in rats administered 50 and 100 mg l^{-1} DMA were significantly less than in control rats. The mean water intake values were 25±3 ml/rat per day.

Figure 1(A) shows the IC–ICP–MS chromatogram of five standard arsenic compounds, i.e. arsenite, MMA, DMA, TMAO and AsBe. The retention time of arsenate (43 min) is not shown on the chromatogram. Figure 1(B) shows a chromatogram typical of the urine of 100 mg l^{-1} DMA-exposed rats. Arsenite, DMA and TMAO were detected in the urine of DMA-exposed rats. An unidentified arsenic compound (metabolite A), which was eluted immediately after MMA, was also detected on the chromatogram. For confirmation, cation-exchange chromatography was used instead of anion-exchange chromatography as a separation method to detect arsenic compounds. The results of anion- and cation-exchange columns were in good agreement.[19] Arsenite, DMA, TMAO and metabolite A were also detected in the feces of DMA-exposed rats.

BBN did not appear to affect the metabolism of arsenic, since all the above metabolites were detected in the urine and feces of both BBN-treated and non-treated rats.

Figure 2(A) shows the relationship between DMA dose and content of arsenical metabolites in urine at the end of 30 weeks. DMA was the main arsenic component in the urine, and increased linearly with dose of DMA. TMAO also increased with dose of DMA. The levels of arsenite and metabolite A were elevated when the dose of DMA was larger than 25 mg l^{-1}. The total arsenic concentration in the urine of 100 mg l^{-1} DMA-exposed rats was 37 mg As l^{-1}, consisting of 10% arsenite, 8% metabolite A, 52% DMA and 30% TMAO.

Figure 2(B) shows the relationship between DMA dose and content of arsenical metabolites in the feces at the end of 30 weeks. The concentration of DMA, arsenite and metabolite A

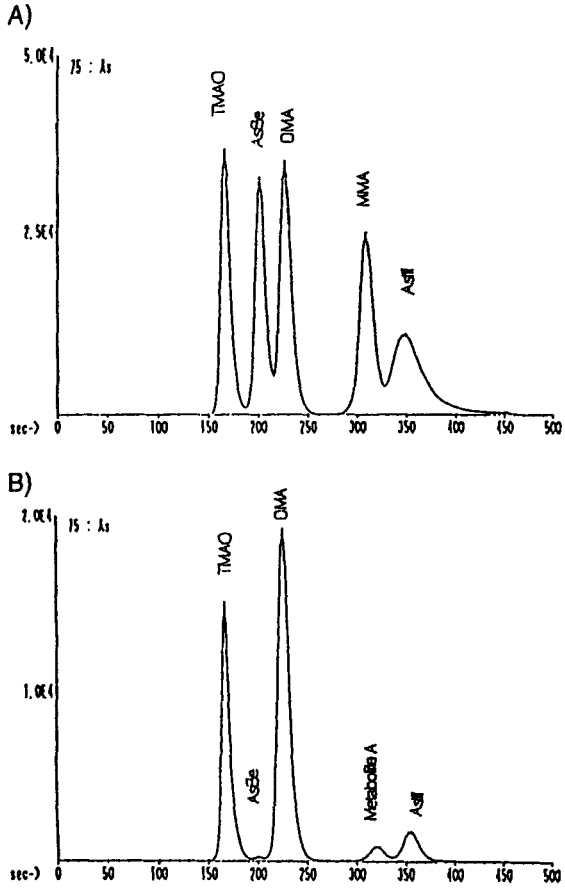

Figure 1 Chromatograms of arsenic compounds: (A) five standard arsenic compounds; (B) arsenic compounds in urine of rat chronically exposed 100 mg l^{-1} DMA.

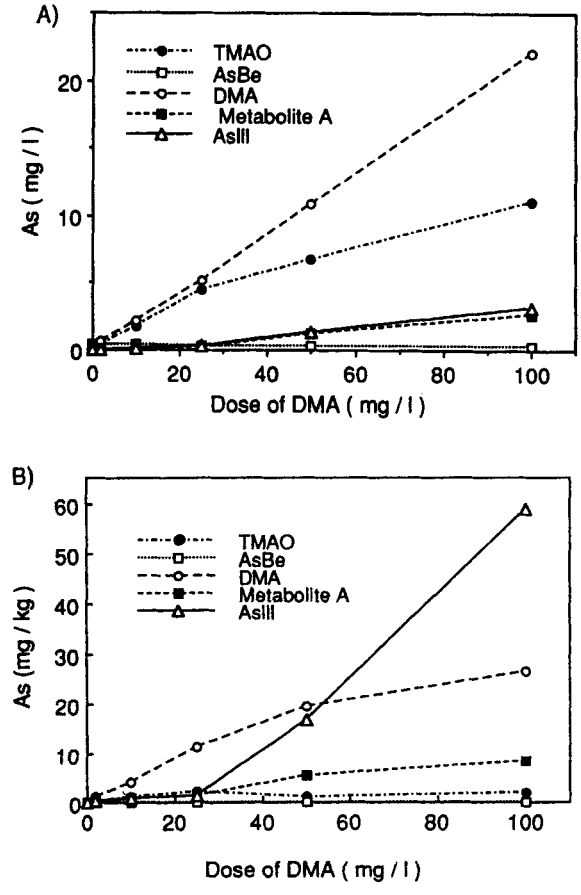

Figure 2 Dose response to arsenic compounds at the end of 30 weeks of oral administration of DMA: (A) in urine; (B) in feces.

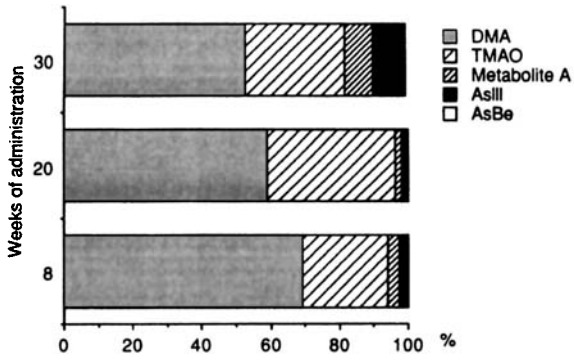

Figure 3 Variation with time of the proportion of five arsenic compounds in the total arsenic in urine following oral administration of 100 mg l^{-1} DMA.

in the feces increased as the dose of DMA increased. Arsenite was significantly elevated when the dose of DMA was larger than 25 mg l^{-1} and was the main arsenic component in the 100 mg l^{-1} dose group. The total arsenic concentration in the feces of 100 mg l^{-1} DMA-exposed rats was 98.95 mg l^{-1}, consisting of 63.1% arsenite, 8.9% metabolite A, 26.2% DMA and 1.8% TMAO. TMAO concentration was low at any dose of DMA.

Figure 3 shows the proportion of five arsenic compounds in the urine at 8, 20 and 30 weeks in 100 mg l^{-1} DMA-exposed rats. DMA content decreased with time, while arsenite and metabolite A excretion were highest at 30 weeks. Only traces of AsBe were found in the urine of DMA-treated rats, as with non-treated rats.

DISCUSSION

In this study we used a combined system of IC–ICP–MS for determination of arsenic compounds. The detection limits for the five arsenic compounds, arsenite, DMA, metabolite A, TMAO and AsBe, were from 1.33 to 4.05 μg l^{-1} as elemental arsenic. The relative standard deviation (RSD) was less than 3% for all arsenic compounds.[19]

Arsenite, metabolite A, DMA, TMAO and AsBe were detected by IC–ICP–MS in the urine and feces of rats after chronic exposure to DMA. AsBe was found as a trace, and it seems to have come from the commercial feed. Metabolite A was eluted immediately after MMA on the chromatogram, but it has not yet been identified. It might be an intermediate metabolite of DMA or a conjugation complex which was formed in the process of excretion of DMA or its metabolites. Arsenite was observed in the urine and feces. This indicates that DMA can be demethylated to inorganic arsenic in rats. However, it has been reported that, so far, DMA could not be demethylated to inorganic arsenic *in vivo*.[10,11] Stevens *et al.*[10] concluded that DMA was not demethylated to inorganic arsenic since they detected no significant differences in the distributions of ^{14}C and ^{74}As in tissues after a single intravenous dose of ^{14}C- and ^{74}As-DMA to rats. However, their result showed that the $^{14}CO_2$ derived from the elimination of ^{14}C-DMA kept rising in the 24 hours after oral administration to rats. It may be that some methyl groups were released from ^{14}C-DMA by demethylation, and were excreted as $^{14}CO_2$ in expired air. Yamauchi and Yamamura[8] reported that inorganic arsenic was detected by atomic absorption spectrophotometry in the urine and feces after oral administration of DMA to hamsters, (after subtraction of the background values and the inorganic arsenic content of the administered DMA). Shiomi *et al.*[20] reported that MMA was detected by HPLC–ICP in the urine after oral and intravenous administration of arsenosugar. However, neither of these reports explain the origin of the arsenite and MMA.

In this study, fecal concentration of arsenite was significantly greater than urine concentration. It increased from a dosage of 25 mg l^{-1}, and the highest fecal content of arsenite was in the 100 mg l^{-1} dose group. On the other hand, TMAO fecal concentration was significantly less than urine concentration and, in any dose group, was very low. From these results, it seems that intestinal bacteria may play a significant role in the demethylation of organic arsenic. Demethylation of DMA by microbes in the estuarine system has been demonstrated by Sanders,[21] and Quinn and Mcmullan[22] demonstrated cleavage of the carbon–arsenic bond of arsenoacetate by Gram- negative bacteria. Stevens *et al.*[10] reported that more DMA was retained in the body after oral administration of DMA than after either intravenous or intratracheal administration of DMA. Furthermore, in the rat, an approximately 10-fold higher level of $^{14}CO_2$ was eliminated following oral administration when compared with other routes. This might also be due to demethylation of DMA by intestinal bacteria in rats.

Body weight gain was affected by DMA, although DMA is considered to be less toxic than inorganic arsenic. This effect may be attributable to arsenite which might be produced by demethylation of DMA in rats, and the effect of DMA in high dosage might be due to accumulation of arsenite during DMA metabolism.

In conclusion, arsenite, DMA, TMAO and metabolite A were detected by IC–ICP–MS in the urine and in the feces of DMA-exposed rats. The results suggest that, besides undergoing further methylation, DMA can be demethylated to inorganic arsenic in rats. The demethylation process of DMA may be associated with intestinal bacteria.

Acknowledgement The authors are deeply indebted to Dr K. Kuroda for helpful discussion. The authors also acknowledge Y. Date, Yokogawa Analytical Systems Inc. (Tokyo, Japan) for her excellent assistance in the measurement of arsenic compounds by IC–ICP–MS.

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