potent in a DNA nicking assay and in a single cell gel assay (Mass et al., 2001).

Yoshida et al. (1998) detected two unidentified metabolites, M-1 and M-2, in urine of rats after long-term oral administration of DMA, MMA, arsenite, or trimethylarsine oxide (TMAO). The amounts of M-1 and M-2 eliminated were higher in DMA-exposed rats than in rats treated with the other arsenicals. Another unidentified metabolite, M-3, was detected only in feces. These unidentified arsenic compounds might be produced in the intestinal tract since the production of M-1 or M-2 was larger after oral administration of DMA than after intraperitoneal injection (Yoshida et al., 2001).

Escherichia coli A3-6 was isolated from cecum of a rat chronically exposed to oral DMA. *E. coli* A3-6 produced M-2 and M-3 from DMA and M-1 from TMAO in GUM medium. A3-6 required Cys to metabolize DMA or TMAO to the unidentified compounds in bouillon medium. The retention time of M-2 in urine of DMA-exposed rats was consistent with M-2 in the sample obtained from the culture mixtures of DMA, Cys, and *E. coli* A3-6 on both cation- and anion-exchange LC-ICP-MS (liquid chromatography with inductively coupled plasma mass spectrometry) chromatograms. These results indicated that urinary M-2 in the rat was the same compound as M-2 produced by *E. coli* A3-6 (Kuroda et al., 2001; Yoshida et al., 2001). The chemical properties of M-2 were studied by LC-ICP-MS and LC/MS analyses. The molecular weight of M-2 was 154 Da and it was a sulfur-containing compound (Yoshida et al., 2003). The molecular weights of M-1 and M-3 were 152 and 170 Da, respectively. The molar ratios of As to S in M-1 and M-3 were confirmed to be 1:1 and 1:2, respectively (unpublished data).

Wanibuchi et al. (1998) suggested that the unidentified metabolite, M-2, might play an important role in the urinary bladder carcinogenesis induced by arsenic in rats since rats with urinary bladder cancer excrete a large amount of M-2 in urine (Li et al., 1998).

In the present study, the cytogenetic effects of DMA or TMAO metabolized by *E. coli* A3-6 were examined. We report that the metabolite M-2 is one of the most toxic arsenic compounds.

Materials and methods

Reagents. Sodium arsenite, sodium arsenate, MMA, DMA, and TMAO were obtained from Tri Chemical Lab. (Yamanashi, Japan). Iododimethylarsine for standard solution of DMAIII was obtained from Dr. W.R. Cullen (University of British Columbia, Vancouver, Canada). Iododimethylarsine in water is hydrolyzed to DMAIII (Mass et al., 2001). Bouillon medium was purchased from Nissui (Tokyo, Japan). Leibovitz-15 (L-15) medium was purchased from SIGMA-ALDRICH, Japan. Fetal bovine serum was obtained from ICN Biochemicals (Costa Mesa, CA, USA). Giemsa's solution was obtained from Merck, Darmstadt, Germany. Trypsin was purchased from Difco (Michigan, USA). Other chemicals (analytical grade) were obtained from Wako Pure Chemical Industry (Osaka, Japan).

Preparation of crude metabolized solution of arsenic compounds. *E. coli* A3-6 was precultured overnight in bouillon. The bacteria collected by centrifugation were suspended in 0.01 M phosphate-buffered saline (pH 7.4) of equal volume to the bouillon medium. To obtain metabolized solution of arsenic compounds, 1 mM of DMA or TMAO and various concentrations of Cys were added to the *E. coli* A3-6 suspension. After incubation at 37 °C for 1 or 6 h under aerobic condition, bacteria in the solutions were removed by centrifugation and filtration through an Ultrafree-MC (Millipore, MA, USA) with a cut-off value of 10 kDa, and the bacteria-free solutions were stored at −80 °C and used for experiments within 2 weeks. We confirmed that contents of arsenic compounds in the solutions were not changed in 1 month.

Cells and methods for cytogenetic study. V79 cells, which originated from Chinese hamster lung, were obtained from the Institute for Fermentation (Osaka, Japan). Cell number measured by hemocytometry after the cells were harvested. Vitality of cells was examined by staining with trypan blue.

To determine sister chromatid exchange (SCE), approximately 1×10^4/ml of V79 cells were plated in 35-mm-diameter Petri dishes with 5 ml L-15 medium and cultured for 24 h at 37 °C in a 5% CO_2 atmosphere. The medium was supplemented with 7% heat-inactivated fetal bovine serum and kanamycin sulfate (50 μg/ml). Various concentrations of metabolized solution of arsenic compounds and 1 μg/ml final concentration of 5-bromodeoxyuridine (BrdU) were added and the cells were cultured in the dark at 37 for 28 h in a 5% CO_2 atmosphere. Colcemid was not added except in the control experiments to avoid its mitotic blocking effect and to determine the net index of metabolized solution treatment. The cells were treated with a hypotonic solution of 0.075 M KCl and fixed with methanol–acetic acids (3:1). Metaphase figures were stained with 0.1 μg/ml Hoechst 33258, irradiated with a black lamp (15 W, 2 cm, 20 min) with SSC (0.3 M sodium chloride + 0.03 M citrate) and stained with 2% Giemsa's solution. The SCEs were counted in 50 metaphases.

For chromosome aberration experiments, the cells were exposed to metabolite solution without BrdU. The mitotic figures were stained with Giemsa's solution. When sister chromatids of a chromosome were separated from each other, mitotic figures were determined as c-mitosis. Two plates were used for a group in the experiments. Results are shown as averages of two plates. Data were statistically analyzed using Student's *t* test or chi-square test.

Liquid chromatography with inductively coupled plasma mass spectrometry. A Model HP4500 ICP-MS (Hewlett-Packard, DE, USA) was used for arsenic detection. The operating conditions for ICP-MS were established in accor-

dance with those reported by Inoue et al. (1994). A Model IC (Yokogawa Analytical Systems, Tokyo, Japan) was used for separating arsenic species. For separations of arsenic compounds, two separation modes, cation and anion exchange, were used. The cation mode experiment, using a Shodex RSpak NN-614 column (150 × 4.6 mm i.d.) packed with cation-exchange resin (Showadenko, Tokyo, Japan), was performed under the following conditions: mobile phase 5 mM HNO_3–6 mM NH_4NO_3, flow rate 0.8 ml/min, and injection volume 50 µl. The anion mode experiment, using an Excelpak ICS-A13 column (75 × 4.6 mm i.d.) packed with anion-exchange resin (Yokogawa Analytical Systems), was performed under the following conditions: mobile phase 3 mM NaH_2PO_4 at pH 6 with NaOH, flow rate 0.8 ml/min, and injection volume 50 µl. A guard column of the same packing type was used for analysis. An outlet from the separation column was directly connected to the nebulizer of the ICP-MS using an ethylenetetrafluoroethylene tube of 0.3 mm i.d.

Determination of arsenic metabolites by LC-ICP-MS. Stock standard solutions of sodium arsenite, sodium arsenate, MMA, DMA, and TMAO were prepared by dissolving each compound in pure water at a concentration of 100 mg As/l. Standard solution of DMAIII was prepared by dissolving iododimethylarsine in pure water and stirring for 30 min under a nitrogen atmosphere just before use. The final diluted aqueous standard mixtures were prepared from each stock standard just before use. The samples were thawed and diluted 50-fold with distilled water just before measurement by liquid chromatography with inductively coupled plasma mass spectrometry (LC-ICP-MS). To obtain precise measurements, 1 mg/l of germanium solution was used as the internal standard for ICPMS; the internal standard was added to the eluate from LC through a mixing joint before introduction to the ICP-MS. The ICP-MS detection mass was set to *m/z* 75 ($^{75}As^+$), *m/z* 72 ($^{72}Ge^+$), and *m/z* 77 ($^{40}Ar^{37}Cl$). The ion intensity at *m/z* 77 was of diagnostic value only in the examination for the possible occurrence of $^{40}Ar^{35}Cl^+$ interference at *m/z* 75. This method was linear in the range of 0.001–10 mg As /l, and the reproducibility (RSD) for 0.01 mg As/l standard arsenic compound was about 2%.

Results

Arsenic compounds in metabolized solution of DMA

The effect of Cys on the production of DMA metabolites was studied by LC-ICPMS. DMA (1 mM) was incubated with various concentrations of Cys (1–7 mM) in the presence of *E. coli* A3-6. DMA was not metabolized by the bacteria in the absence of Cys. After a 1-h incubation, The addition of Cys (3 mM) decreased DMA to 32% and increased M-2 to 37%. In the case of Cys concentrations more than 3 mM, M-2 production was decreased. A slight amount of DMAIII appeared at 1 mM Cys (1.2%) or 3 mM Cys (6.9%). Cys (1–5 mM) increased M-3 in a concentration-dependent fashion. An unknown peak (30%) appeared with 7 mM Cys (Fig. 1A). After 6-h incubation, Cys (7 mM) decreased DMA to 0% and increased M-3 to 99.5%. M-2 concentration was highest (37%) at 1 mM Cys. DMAIII and the unknown peak disappeared (Fig. 1B). The cytotoxic and genotoxic effects of the DMA metabolites produced by 6 h of incubation were then studied using V79 cells.

Cytotoxicity of metabolized solution of DMA

V79 cells were exposed for 28 h to DMA metabolites produced after a 6-h incubation with various concentration

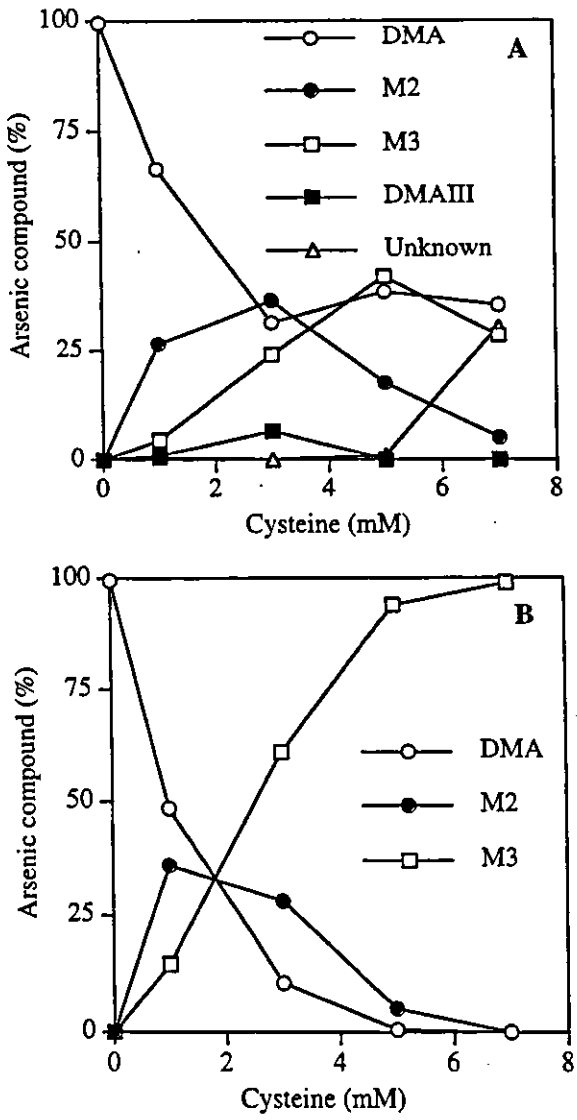

Fig. 1. Arsenic compounds in metabolized solution of DMA by *E. coli* A3-6 with various concentration of Cys. DMA 1mM and *E. coli* A3-6 were incubated in phosphate buffer with various concentration of Cys at 37 °C. (A) incubated for 1 h; (B) incubated for 6 h.

of Cys and cell survival was determined. The metabolites produced from a solution of 1 mM DMA and 1 mM Cys exhibited the strongest cytotoxicity and the metabolite solution produced by mixing DMA1 mM, and Cys 0 or 7 mM did not exhibit any toxic effect when used at 50 μM total As (Fig. 2). Amount of metabolite solution for 50% reduction of survival (IC_{50}) was calculated from Fig. 2. The IC_{50} values were 2.08, 4.03, and 10.71 μM As for Cys 1, 3, and 5 mM, respectively.

Information on toxic substance in a mixture is usually obtained by correlation between content of each substance and toxicity of the mixture. IC_{50} of the solutions was linearly correlated with content of M-2 ($r = 0.999$, $P = 0.025$, Fig. 3) and the contents of DMA or M-3 did not exhibit any correlation with cytotoxicity of the microbially metabolized solution (DMA; $r = 0.806$, $P = 0.403$, M3; $r = 0.918$, $P = 0.259$). These results strongly suggest that the cytotoxic substance in the metabolized solution of DMA was M-2.

Effects of DMA metabolite solution on mitosis

Chromosomal preparations of the V79 cells exposed for 28 h to the DMA metabolite solution produced by a 6-h incubation in the presence of various concentration of Cys were studied under a microscope. All the DMA metabolite solutions produced with Cys increased the numbers of mitotic cells in a concentration-dependent fashion (Fig. 4). The metabolites resulting from the incubation of

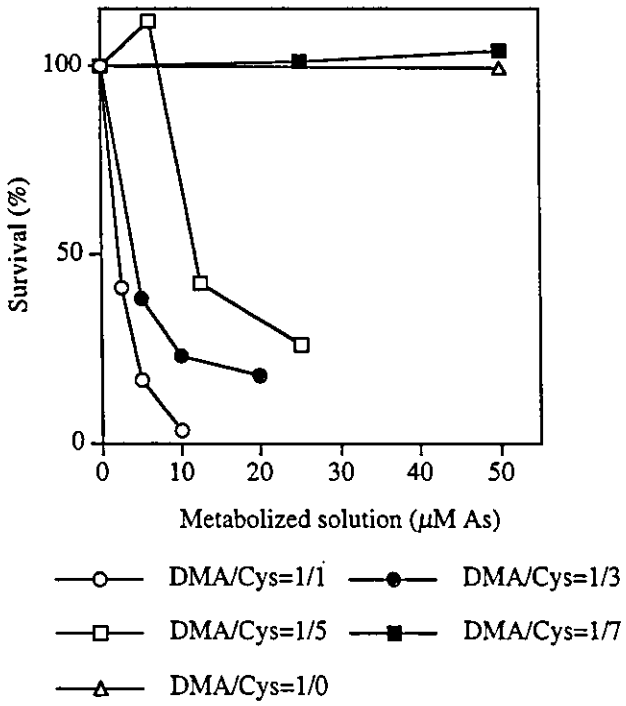

Fig. 2. Cytotoxicity of 6-h-incubated metabolized solution of DMA by *E. coli* A3-6 with various concentrations of Cys. V79 cells were treated with the solutions for 28 h. Survival was the average of two plates.

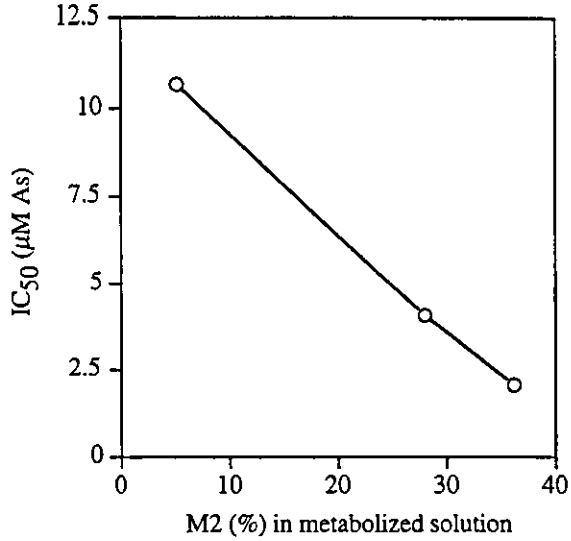

Fig. 3. Relationship between IC_{50} and M-2 content of 6-h-incubated metabolized solution of DMA by *E. coli* A3-6 with various concentrations of Cys. As concentration of IC_{50} was calculated from Fig. 2.

DMA (1 mM) and Cys (1 mM) were the strongest in increasing mitotic cells and mitotic cells became fewer as Cys concentration was increased. The DMA metabolite solution at Cys (1 mM) increased the frequency of mitotic cell to 4.4% at 10 μM As, which was more than twice the control rate (2.0%). At this concentration, the survival

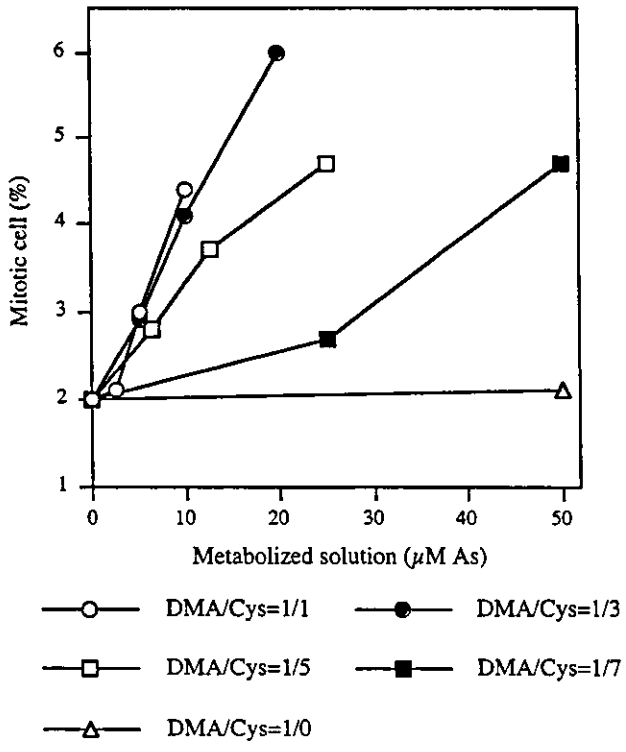

Fig. 4. Effects of 6-h-incubated metabolized solution of DMA by *E. coli* A3-6 with various concentrations of Cys on mitosis of V79 cells. V79 cells were treated with the solutions for 28 h. Frequency of mitotic cell was the average of two plates.

decreased to 4% of the control level. Mitotic figures were not observed at 20 μM As. These results suggested that the DMA metabolite solution caused mitotic arrest. The DMA metabolite solution produced without inclusion of Cys did not increased mitotic cells even at 50 μM As.

Amount of each DMA metabolite solution required for doubling the frequency of mitotic cells was calculated from Fig. 4. The values were 8.7, 9.6, 16.3, and 41.3 μM As for DMA metabolite solutions produced by incubation with 1, 3, 5, and 7 mM Cys, respectively. The value was exponentially correlated with M-2 content as shown in Fig. 5 (r = 0.999, P = 0.0008) and was not correlated with M3 or DMA (M3; r = 0.691, P = 0.309, DMA; r = 0.979, P = 0.128).

Induction of tetraploid by DMA metabolite solution

More than 90% of the mitotic figures in the control had 22 chromosomes and a mitotic figure of which chromosome number was 40–46 was determined as a tetraploid. The DMA metabolite solutions produced with Cys (1–5 mM) induced tetraploids in a concentration-dependent fashion (Fig. 6). The metabolites produced by incubation of DMA (1 mM) and Cys (1 mM) were the most active in this regard. Tetraploid in the control cells was 1% and in those of the cells exposed to the DMA metabolite solution produced with Cys (1 mM) at 2.5, 5, and 10 μM As were 10%, 14.9%, and 25.5%, respectively. The increases in tetraploids became weaker as the Cys concentration used to produce DMA metabolites was increased. The frequency of tetraploid cells appeared to decrease when the ratio of mitotic cells increased over 4%, twice that of control. The DMA metabolite solutions produced with 0 or 7 mM Cys did not induce tetraploidy at 25 or 50 μM As. These results suggested that M-2 in the DMA metabolite induced tetraploidy as well as mitotic arrest.

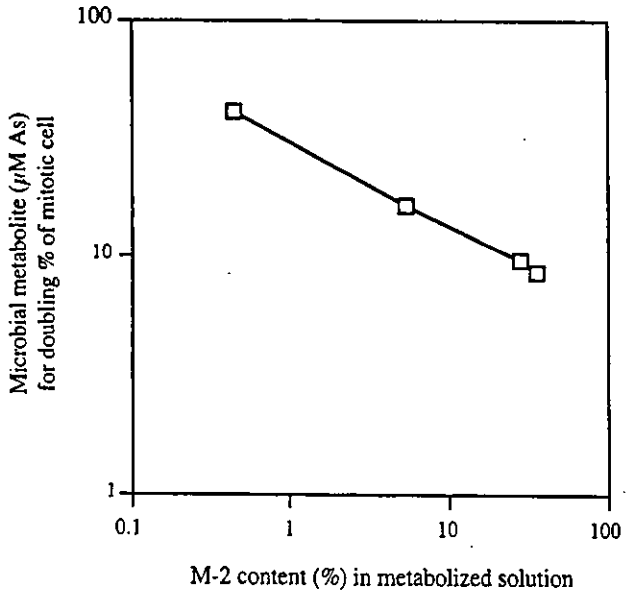

Fig. 5. Relationship between 6-h-incubated metabolized solution of DMA by *E. coli* A3-6 for doubling frequency of mitotic cell and M-2 content in metabolite solution. As concentration of metabolite was calculated from Fig. 4.

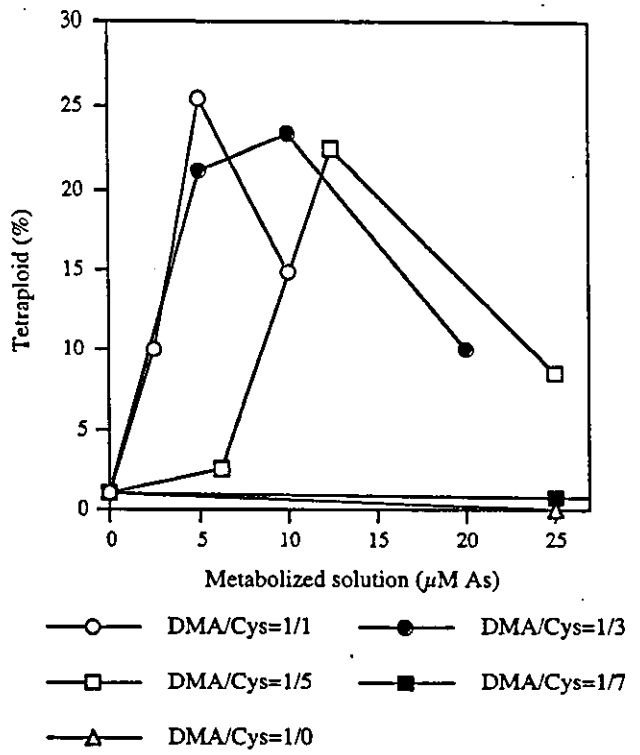

Fig. 6. Effects of 6-h-incubated metabolized solution of DMA by *E. coli* A3-6 with various concentrations of Cys on induction of tetraploid in V79 cells. V79 cells were treated with the metabolite for 28 h. Frequency of tetraploid was the average of two plates.

Induction of chromosomal aberration by DMA metabolite solution

Chromosomal aberrations were observed in the mitotic figures exposed to the microbial metabolite solution of DMA (1 mM) in the presence of Cys (1 mM). These results were summarized in Table 1. The rate of aberrant cells in the controls was 2%. The DMA metabolite solution at 5 μM As significantly increased the frequency of aberrant cells to 27%, 3-fold more than that induced by the DMA metabolite reduced by DMA alone at 100 μM As. Chromatid gaps were the most frequent aberration. Aberrant cells reached a rate 70% at 10 μM As of DMA metabolite. Chromatid breaks and chromatid gaps were the main types of aberrations. Mitomycin C, which was used for positive control, induced aberrant cells (34%) at 0.03 μM. The main types of aberrations here were gaps and chromatid exchanges.

c-Mitosis is considered an index of mitotic poisoning. More than 90% of the mitotic figures treated with the metabolized solution 5 and 10 μM As were c-mitosis. c-

Table 1
Chromosomal aberrations in V79 cells treated with metabolized solution of dimethylarsinic acid by *E. coli* A3-6 for 28 h

Chemical	c-mitosis (%)	Type of aberration					Total
		ctg	ctb	cte	csg	dic	
Control[a] 1/10 (v/v)	–[b]	1/1	0/0	0/0	1/1	0/0	2/2
Control[c] 100 μM As	71	7/8	2/3	0/0	0/0	0/0	9*/11*
Metabolized[d] solution 5 μM As	94	22/31	8/10	0/0	0/0	1/1	27**/42**
Metabolized[d] solution 10 μM As	97	54/149	54/113	1/1	0/0	0/0	70**/263**
MMC[e] 0.03 μM	–[b]	18/21	8/8	5/8	0/0	0/0	28**/34**

One hundred metaphases per group were observed. left/right, aberrant cell/ chromosomal aberration; ctg, chromatid gap; ctb, chromatid break; cte, chromatid exchange; csb, chromosome break.
[a] Control was prepared with metabolized solution of A3-6 and Cys 1 mM and was added to V79 culture with 1/10 volume of culture medium.
[b] Treated with colcemid 0.1 μg/ml.
[c] Control was prepared with metabolized solution of A3-6 and DMA 1 mM.
[d] DMA 1mM and Cys 1mM were incubated with A3-6 for 6 h.
[e] Mitomycin C.
* $P < 0.05$, significantly higher than Control [1/10 (v/v)].
** $P < 0.001$, significantly higher than Control [1/10 (v/v)] and Control (100 μM As).

Mitosis was 7% in the control, whereas that of the metabolized solution without Cys was 71% at 100 μM As.

Induction of SCE by DMA metabolite solution

The DMA metabolite solution produced by incubation of DMA (1 mM) and Cys (1 mM) induced SCE in the range of 1 and 8 μM As (Table 2). It induced 0.60 ± 0.21 SCE per chromosome at 8 As μM in a dose-dependent manner. SCE at 16 μM As were not counted because the solution exhibited serious cytotoxic effects and most mitotic figures were once divided. Mitomycin C, which was used as a positive control, induced the same level of SCE (0.66 ± 0.20) at 0.003 μM.

Chromosomes per cell also increased in a concentration-dependent manner with the microbial DMA metabolite. Distribution of chromosome number in a mitotic figure of which sister chromatids were differentially stained is shown in Fig. 6. Mitotic figures in 94% of control had 22 chromosomes, and other mitotic figures (6%) were within one or two chromosomes of 22. A mitotic figure, which had 20–21 or 23–41 chromosomes, was determined as an aneuploid. Of the mitotic figures treated with the DMA metabolite solution (4 μM As), tetraploids were 6% and aneuploids were 8%, which were within one chromosome of 22. Tetraploids increased to 20% at DMA metabolite arsenic levels of 8 μM As and aneuploids increased to 12%. However, the increase of aneuploids by treatment of the microbial metabolite was not significant. These results suggest that the microbial metabolite of DMA specifically induced tetraploid (Fig. 7).

Table 2
SCE induction in V79 cells treated with metabolized solution of dimethylarsinic acid by *E. coli* A3-6 for 28 h

Metabolized solution (As μM)	SCE per cell	SCE per chromosome	Chromosome per cell
Control[a] 1/10 (v/v)	5.3 ± 2.4	0.24 ± 0.11	22.0 ± 0.4
Control[b] 10	5.3 ± 2.3	0.24 ± 0.10	22.1 ± 1.4
1	7.6 ± 3.3*	0.35 ± 0.15*	22.0 ± 0.3
2	9.2 ± 2.9*	0.42 ± 0.13*	22.1 ± 0.7
4	11.8 ± 4.9*	0.51 ± 0.19*	23.4 ± 5.3
8	16.2 ± 8.1*	0.60 ± 0.21*	26.8 ± 8.9*
MMC 0.03 μM	14.4 ± 4.4*	0.66 ± 0.20*	21.9 ± 0.3

SCE was counted in 50 metaphases of each groups. The metabolized solution was prepared after 6-h incubation of DMA 1 mM, Cys 1 mM, and *E. coli* A3-6. The metabolized solutions were prepared with DMA 1 mM, Cys 1 mM, and A3-6. MMC, mitomycin C.
[a] Control was prepared with metabolized solution of A3-6 and Cys 1 mM and was added to V79 culture with 1/10 volume of culture medium.
[b] Control was prepared with metabolized solution of A3-6 and DMA 1 mM.
* $P < 0.001$, significantly higher than control.

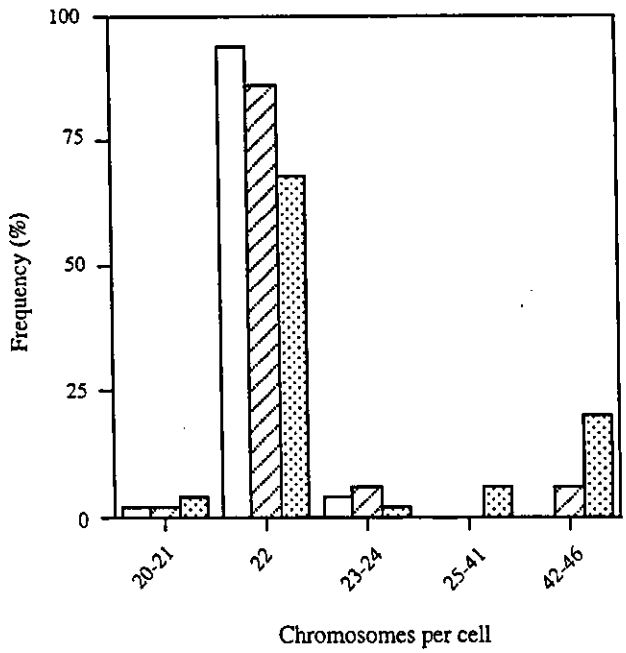

Fig. 7. Chromosome numbers in V79 cells exposed to 6-h-incubated metabolized solution of DMA 1 mM and Cys 1 mM by *E. coli* A3-6 for 28 h. Chromosomes in a mitotic figure, of which sister chromatids were differentially stained, were counted. White columns, control; striped columns, 4 μM As; dotted columns, 8 μM As of the solution.

Table 3
Toxicity of metabolized solution of V79 cells treated with metabolized solution of trimethylarsine oxide by *E. coli* A3-6 for 28 h

Metabolite (As mM)	M-1 (%)	Survival (%)	Mitotic cell (%)	Tetraploid (%)	SCE/cell	Aberrant cell (%)
Control[a] 1/10 (v/v)	0.0	100.0	2.9	1.5	4.8 ± 1.9	2
Control[b] 0.1	0.0	103.5	2.4	2.5	5.6 ± 2.0	1
0.1	96.8	102.9	3.5	2.0	5.5 ± 2.6	2

The metabolized solution of trimethylarsine oxide was prepared after 6-h incubation of TMAO 1 mM, Cys 1 mM, and *E. coli* A3-6.
[a] Control was prepared with metabolized solution of A3-6 and Cys 1 mM and was added to V79 culture with 1/10 volume of culture medium.
[b] Control was prepared after 6-h incubation of TMAO 1 mM and A3-6.

Effects of TMAO metabolite solution in V79 cells

E. coli A3-6 metabolized 96.1% of TMAO (1 mM) in the presence of Cys (1 mM) after incubation for 6 h, whereas the rest remained as unchanged TMAO. The TMAO metabolite solution did not exhibit cytotoxic or genotoxic effect in V79 cells at 0.1 mM As (Table 3).

Discussion

DMAIII found in metabolite solutions (Fig. 1A) might not be due to bacterial action because DMAIII is found in a mixture of DMA and Cys without bacteria (Zhou et al., 2003). The M-2 content in the 6-h-incubated metabolized solution was highest when DMA and Cys was equimolar. M-3 content increased with the concentration of Cys (Fig. 1). This result is reasonable because M-2 contains only one sulfur moiety (Yoshida et al., 2003) and M-3 contains two sulfur moiety (unpublished data). There are no reports of the direct participation of *E. coli* in the production of arsenothiol compounds. However, Tsao and Maki (1991) indicated that the reactive derivative, $(CH_3)_2AsSR$, was formed after the reduction of DMA by a thiol and the derivative bound to *Eco*RI methyl transferase by mercaptide exchange with a Cys residue located close to a tryptophan site.

The DMA metabolite solution produced with Cys was highly cytotoxic and M-2 was the probable main toxicant. M-2 content was linearly correlated with IC_{50} of the DMA metabolite solutions produced with various Cys concentrations (Fig. 3). However, IC_{50} of the microbial metabolite solution should be greater than 0 μM As, when M-2 content is extrapolated 100% and IC_{50} of DMA is about 1 mM in V79 cells (Kitamura et al., 2002), that is, M-2 content is 0%. Then a point (M-2 % = 0, IC_{50}= 1000 μM As) was added in Fig. 3. The relationship between M-2 content and IC_{50} appeared to be exponential as shown in the relationship between the value for doubling mitotic cell and M-2 content (Fig. 4).

It is important to compare our results with the microbial metabolites of DMA with those of trivalent arsenic because trivalent arsenic is more toxic than pentavalent. Regardless of the cell types, trivalent monomethyl arsenicals are more cytotoxic with IC_{50} values ranging from 0.4 to 5.5 μM. DMAIII derivatives are as cytotoxic as MMAIII species and more cytotoxic than arsenite in most cell types (Cohen et al., 2002; Petrick et al., 2000; Styblo et al., 2000, 2002). IC_{50} 5.4 μM of arsenite for V79 cells was estimated with the same method in the present paper (Eguchi et al., 1997).

Yamanaka et al. (1989) reported that volatile trivalent metabolites, dimethylarsine and trimethylarsine, were detected in the gas phase after DMA was added to cell suspensions of *E. coli* B in sealed tube. It is possible that the microflora present in the ceca are responsible for metabolism of arsenic compounds. The toxicity of complex of DMAIII with GSH (DMAIIIGS) was reported to be higher than that of DMA (Vega et al., 2001). Styblo et al. (1997) reported that complexes of MMAIII with GSH (MMAIII(GS)2) or with Cys (MMAIII(Cys)2) were the most potent inhibitors of yeast GSH reductase. These results strongly suggest that M-2 may be a trivalent sulfur-containing arsenic compound.

The DMA metabolite solution produced by incubation of DMA (1 mM) and Cys (1 mM) strongly induced c-mitosis, mitotic arrest, and tetraploid at μM As levels (Table 1, Figs. 4 and 5). This suggests that the microbial metabolite was a strong mitotic poison. DMA is also a mitotic poison but it is only active at millimolar concentration (Eguchi et al., 1997; Endo et al., 1992). DMA inhibits normal assembly of tubulin in vitro, induces multipolar spindles in mitotic cells (Kawata et al., 2001), and also inhibit tubulin's GTPase activity (Kawata et al., 2000). It is of interest whether the microbial metabolite of DMA exhibits the same activity as DMA.

Sodium arsenite induces SCE and chromosomal aberrations in different cell types. The aberrations found in many studies are chromatid gaps, fragmentations, endoreduplications, and chromosomal breaks (Gebel, 2001). However, tetraploidy induced by the DMA metabolite solution did not originate from endoreduplication. Sodium arsenite induces significant increases in chromosomal aberrations in human fibloblasts at 3.8 and 7.7 μM (Oya-Ohta et al., 1996). These results suggest that genotoxicity of the microbial metabolite with equimolar DMA and Cys was as strong as arsenite. We reported that M-2-induced cytotoxicity was decreased by the addition of SOD (Yoshida et al., 2003). This finding suggests that the production of reactive oxygen species may play an important role in cytotoxicity and the oxidative DNA damage by M-2.

Yoshida et al. (1998) reported that M-2 concentration in urine of rats administered DMA 100 mg/l As via drinking water for 7 months was 105 μM As. They suggested that M-2 was not produced through the metabolism of the rats because M-2 concentration was not affected by glutathione

depletion, and that the amount of elimination of M-1 and M-2 after intraperitoneal administration was less than after oral administration (Yoshida et al., 2001). Wei et al. (1999, 2002) observed urinary bladder tumors in rats after exposure to DMA at 200 and 50 ppm via drinking water and did not observe tumors in other organs. The DMA metabolite solution produced by incubation of DMA (1 mM) and Cys(1 mM) induced chromosomal aberrations and SCE at 5–10 μM As. These results strongly suggest that M-2 concentration in urine of rats produced by administration of an amount of DMA via drinking water known to cause bladder tumors is sufficient to exhibit genotoxic effects in the urinary bladder.

Some arsenical metabolism takes place in the gut, with the involvement of the associated microorganisms. Cullen et al. (1989) showed that homogenates of mouse ceca, which are sites of high microbiological activity, can methylate methylarsine oxide to dimethylarsinate or demethylated it to arsenate. We propose here a hypothesis that intestinal bacteria play an important role in carcinogenicity of DMA.

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Genotoxicity of dimethylarsinous acid: high induction of tetraploids

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Arsenic is a carcinogen in humans. However, neither the mechanism of action nor the ultimate chemical form of arsenic which causes cancer has been clearly defined. Dimethylarsinous acid is detected in the urine of individuals who ingest arsenic-polluted drinking water. The cytogenetic study in V79 cells using iododimethylarsine, which is easily hydrolyzed to dimethylarsinous acid in water, revealed that dimethylarsinous acid was very cytotoxic (50% growth inhibition concentration; 1.1 ± 0.14 μM), and either induced aneuploids or a high rate of tetraploids (73% at 2.5 μM). Dimethylarsinous acid caused mitotic arrest, since the mitotic index at toxic dose (5 μM) was 13.9%, significantly higher than the control (2.7%). Dimethylarsinous acid significantly increased sister chromatid exchange (SCE) and chromosomal aberrations, most of which were chromatid gaps and chromatid breaks. The cytotoxicity and the activity of dimethylarsinous acid in inducing chromosomal aberration or SCE was as effective as arsenite, but the activity was much lower than that of mitomycin C, which was used as a positive control. The most potent effects of dimethylarsinous acid on the cells were induction of aneuploids, tetraploids and c-mitosis. Our results suggest that toxicity of dimethylarsinous acid is strongly related to the disturbance of the normal cell cycle. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: dimethylarsinous acid (DMA(III)); SCE; chromosomal aberration; tetraploid; aneuploid; mitotic arrest; c-mitosis; V79

INTRODUCTION

When arsenate is introduced into the mammalian body it is reduced to trivalent arsenite, and then it is methylated to monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V)).[1] The acute toxicity of pentavalent organic arsenic compounds is much lower than that of inorganic arsenic.[2] Methylation of arsenic can be considered a mechanism of detoxification.[3,4] DMA(V), a pentavalent organic arsenic, is the major metabolite of inorganic arsenic in humans.[5] DMA(V) has been shown to be a promoter of carcinogenesis[6–8] and a complete carcinogen[9,10] in rats or mice. However, the mechanism by which arsenic compounds cause human cancers is not yet known.[11,12]

Trimethylarsine oxide (TMAO) has been detected in the urine of humans administered DMA(V)[13] or arsenosugar,[14] and in the urine of rats administered arsenite, MMA(V) or DMA(V).[15] Dimethylarsinous acid (DMA(III)) is considered an intermediate between DMA(V) and TMAO, and is detected in the urine of rats exposed orally to DMA(V) for a long time,[16] in the liver of hamsters administered with arsenate,[17] and in the urine of individuals who have ingested inorganic arsenic-polluted drinking water.[18,19] Methylated trivalent arsenicals, such as DMA(III) and monomethylarsonous acid (MMA(III)), which is an intermediate between MMA(V) and DMA(V), are more cytotoxic than arsenite.[20–22] These compounds were shown to be very potent in a DNA nicking assay and in a single-cell gel assay.[23] Recently, Ochi *et al.*[24] reported that DMA(III) is extremely clastogenic. We carried out a cytogenetic study of DMA(III) using V79 cells and iododimethylarsine, which is hydrolyzed in water to DMA(III).[23]

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MATERIALS AND METHODS

Cells and reagents

V79 cells, which originated from Chinese hamster lung, were obtained from the Institute for Fermentation (Osaka,

Japan). Leibovitz-15 (L-15) medium was purchased from Sigma-Aldrich, Japan. Fetal bovine serum was obtained from ICN Biochemicals, Costa Mesa, California. Mitomycin C (MMC), Hoechst 33258 and 5-bromodeoxyuridine (BrdU) were purchased from Wako Pure Chemicals, Osaka, Japan. Giemsa's solution was obtained from Merck, Darmstadt, Germany. Trypsin was purchased from Difco, Michigan. Iododimethylarsine was obtained from W. R. Cullen, University of British Columbia, Canada.

Methods

DMA(III) solution was freshly prepared by dissolving iododimethylarsine in water. In water, this iodocompound produces DMA(III) quickly. For sister chromatid exchange (SCE) experiments, approximately 4×10^4 ml^{-1} of V79 cells were plated in 35 mm diameter Petri dishes with 5 ml L-15 medium and cultured for 24 h at 37 °C in a 5% CO_2 atmosphere. The medium was supplemented with 7% heat-inactivated fetal bovine serum and kanamycin sulfate (50 μg ml^{-1}). Various concentrations of DMA(III) and 1 μg ml^{-1} final concentration of BrdU were added and the cells were cultured in the dark at 37 °C for 28 h in a 5% CO_2 atmosphere. Colcemid was not added, except in the control experiments in order to avoid its mitotic blocking effect and to determine the net index of DMA(III) treatment. The cells were treated with a hypotonic solution of 0.075 M KCl and fixed with methanol–acetic acid (3:1). Metaphase figures were stained with 0.1 μg ml^{-1} Hoechst 33258, irradiated with a black lamp (15 W, 2 cm, 20 min) in SSC (0.3 M sodium chloride + 0.03 M citrate) and stained with 2% Giemsa's solution. The SCEs were counted in 50 metaphases where possible. The mitotic index was determined as the proportion of metaphase cells in 1000 cells. After the cells were harvested, cell numbers were measured by hemocytometry. The vitality of the cells was assessed by staining with trypan blue. When sister chromatids of a chromosome were separated from each other, the mitotic figure was considered to exhibit c-mitosis.

For chromosomal aberration experiments, the cells were exposed to DMA(III) without BrdU. The mitotic figures were stained with Giemsa's solution. Two plates were used as a group in these experiments. Results are shown as the averages of two plates. Data were analyzed statistically using Student's *t*-test or the χ^2 test.

RESULTS

V79 cells were exposed to various concentrations of DMA(III) for 28 h and viable cell numbers were counted. As shown in Fig. 1, survival decreased linearly over the range of the concentration from 0.625 to 5 μM. At 10 μM, cell survival decreased to less than 10% of the control level. The LC_{50}, the concentration resulting in a 50% decrease in a cell population, was estimated to be 1.1 ± 0.14 μM.

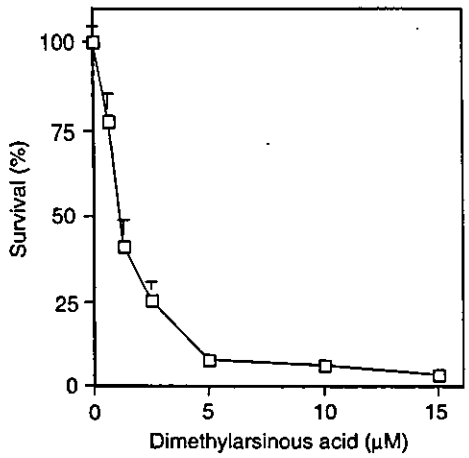

Figure 1. Survival of V79 cells exposed to DMA(III) for 28 h. Error bars are standard deviations ($n = 4$).

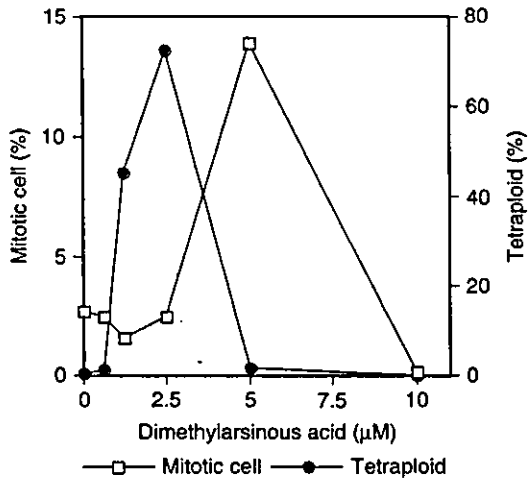

Figure 2. Effects of DMA(III) treatment for 28 h on V79 cells. Mitotic cells (%) were counted by observation of 1000 cells. Tetraploids and c-mitosis were counted in 100 metaphases. Values are the average of two plates. Asterisk indicates significantly higher than the control ($p < 0.001$).

Chromosome preparations of the cells exposed to DMA(III) were produced and observed by microscopy. DMA(III) at toxic concentrations caused increased numbers of mitotic cells (Fig. 2). Mitotic indices were at the same level as the control below concentrations of 2.5 μM DMA(III), but increased to 14% at 5 μM DMA(III), fivefold more than the control level (2.5%). Cell survival decreased to less than 7% of the control level at this concentration. Mitotic figures were not observed at 10 μM. These results suggest that DMA(III) caused mitotic arrest at 10 μM DMA(III).

More than 95% of V79 cells in the control had 22 chromosomes (diploidy). A mitotic figure with 42–46 chromosomes was classified as a tetraploid in this experiment. Tetraploids increased significantly to 45% and 73% at 1.25 μM

and 2.5 μM of DMA(III) respectively, and decreased to the control level at 5 μM (Fig. 2). Tetraploids were not observed in the control cells.

c-Mitosis is considered as a marker of mitotic malfunction. DMA(III) significantly increased c-mitosis linearly between concentrations of 0.625 and 5 μM (Table 1). The level of c-mitosis was observed as 7% in the control, whereas at 0.625 μM of DMA(III) it was 17% ($p < 0.001$). c-Mitosis levels reached 94% at 5 μM DMA(III).

Chromosomal aberrations induced in V79 cells treated with DMA(III) or MMC for 28 h are shown in Table 1. DMA(III) monotonically increased cells with chromosome aberration at concentrations between 1.25 and 5 μM. Percentages of aberrant cells in the control were 2% (gaps included) and 1% (gaps excluded). DMA(III) at 2.5 μM significantly increased the frequency of aberrant cells to 17%. Chromatid gaps were the most frequent aberrations at this concentration. Aberrant cells reached 71% at 5 μM DMA(III). Chromatid breaks, chromatid gaps and multi-aberrations, consisting of many chromatid breaks and chromatid gaps, were the main types of aberration at this concentration. MMC, which was used as a positive control, significantly induced aberrant cells (34%) at 0.03 μM. The main types of aberration caused by MMC were gaps and chromatid exchanges.

Results of SCE induction by DMA(III) are shown in Table 2. SCE per cell increased in a dose-dependent fashion with DMA(III) treatment, as did chromosome number per cell. Consequently, SCE per chromosome was considered a better treatment marker than SCE per cell. DMA(III) significantly and dose-dependently induced the SCE per

Table 1. Chromosome aberrations in V79 cells treated with DMA(III) for 28 h

Concentration (μM)	c-Mitosis (%)	Types of aberration[a] (%)										Aberrant cell (%)
		ctg	ctb	cte	csg	csb	cse	dic	atten	mab	total	
DMA(III)												
0	7	0	0	1	1	0	0	0	0	0	2	2
0.625	17[b]	0	0	0	0	0	0	0	0	0	0	0
1.25	42[b]	13	7	0	0	0	0	0	0	1	21[b]	8
2.5	51[b]	15	2	1	0	2	0	0	0	1	21[b]	16[b]
5.0	94[b]	71	95	5	0	0	0	0	5	20	196[b]	71[b]
MMC												
0.03	—	21	8	8	0	0	0	0	0	0	34[b]	28[b]
0.15	—	73	41	80	2	1	1	1	0	3	195[b]	60[b]

One hundred metaphases per group were observed.
[a] ctg, chromatid gap; ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse, chromosome exchange; dic, dicentric; atten, attenuation; mab, multiple aberration.
[b] Significantly higher than the control ($p < 0.001$).

Table 2. SCEs in V79 cells treated with DMA(III) for 28 h

Concentration (μM)	Metaphase	SCEs ± SD[a]		Chromosomes ± SD (per cell)
		Per cell	Per chromosome	
DMAIII–I				
0	50	5.6 ± 2.5	0.25 ± 0.11	21.9 ± 0.7
0.625	50	9.0 ± 4.5[b]	0.37 ± 0.18[b]	25.1 ± 6.5[b]
1.25	50	19.1 ± 8.6[b]	0.50 ± 0.19[b]	37.9 ± 10.1[b]
2.5	20	27.6 ± 8.9[b]	0.63 ± 0.19[b]	43.8 ± 8.8[b]
MMC				
0.003	50	14.4 ± 4.4[b]	0.66 ± 0.20[b]	21.9 ± 0.3
0.006	50	20.8 ± 4.7[b]	0.95 ± 0.22[b]	21.9 ± 0.4
0.015	50	40.7 ± 9.2[b]	1.86 ± 0.41[b]	21.9 ± 0.7

Fifty metaphases per group were observed where possible. Twenty metaphases were observed for 2.5 μM DMAIII–I, because differentially stained metaphases were decreased in number due to toxicity.
[a] SD, standard deviation.
[b] Significantly higher than the control ($p < 0.001$).

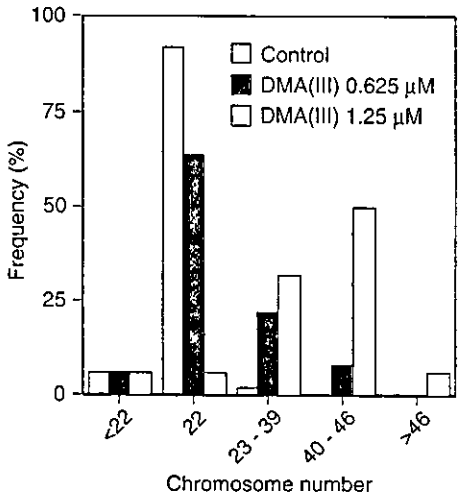

Figure 3. Chromosome numbers in V79 cells exposed to DMA(III) for 28 h. Chromosomes for which sister chromatids were clearly differentially stained were counted.

chromosome between concentrations of 0.625 and 2.5 μM. DMA(III) induced 0.63 ± 0.19 SCEs per chromosome at 2.5 μM. SCEs were counted in only 20 metaphases at this concentration, since DMA(III) exhibited serious cytotoxic effects and most mitotic figures were already divided. MMC, used as a positive control, induced the same level of SCEs (0.66 ± 0.20) at 0.003 μM as that induced by DMA(III) at 2.5 μM.

The mitotic figures in which SCEs were counted were classified into three types: diploidy ($n = 22$), aneuploidy ($n < 22$, $22 < n < 42$) and tetraploidy (Fig. 3). Most mitotic figures in the control were diploids (92%), and the other mitotic figures (8%) were aneuploids within one or two chromosomes of 22. Aneuploids increased significantly to 28% and 44% after exposure to DMA(III) concentrations of 0.625 μM and 1.25 μM respectively. However, DMA(III) did not increase hypoploidy ($n < 22$). The rate of hypoploidy is usually equal to the rate of aneuploids that have recessive chromosomes. In this case the mitotic cells had already passed the DNA synthesis phase twice. Possibly, any cells which had less than 22 chromosomes were unable to undergo DNA synthesis twice due to lack of chromosomes, or did not survive.

DISCUSSION

There are several reports available on the cytotoxicity of trivalent methylated arsenicals.[20–25] Trivalent mono- or dimethylated arsenicals used in such studies were free forms, oxides or complexes with glutathione. Estimated LC_{50} values ranged from 0.8 to 30.8 μM. The cytotoxic effects of trivalent methylated arsenicals appeared to be variable, depending on the chemical form. Styblo *et al.*[20] reported that, in primary rat hepatocytes, the LC_{50} of DMA(III) was 2.7 μM and that of arsenite was 5.1 μM. Rat hepatocytes show a high capacity to methylate arsenicals. Using trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells derived from various tissues, they concluded that high methylation capacity does not protect cells from the acute toxicity of trivalent arsenicals. In our experiments, the LC_{50} of DMA(III) was found to be 1.1 ± 0.14 μM and that of sodium arsenite was 5.4 μM in V79 cells, estimated using the same method as previously described.[26] These values correlate well to those of Styblo *et al.*[20] Our results support their conclusion, since V79 cells do not have a high methylation capacity (data not shown).

Ochi *et al.*[24] observed abnormality, multipolar spindles and aneuploidy in the DMA(III)-treated cells and suggested that DMA(III)-induced multipolar spindles and multipolar division may be associated with the induction of aneuploidy. However, our results showed that induction of tetraploidy occurred at concentrations of DMA(III) where an increase of mitotic cells was not yet observed (Fig. 2). These results suggested that induction of tetraploidy might be due to inhibition of cytokinesis by some mechanism such as inhibition of forming a contractile ring.

Arsenite had been the most toxic and genotoxic among arsenic compounds detected in mammals until trivalent methylated arsenic compounds were detected. It is important to compare our results of DMA(III) with those of arsenite published by others. Arsenite significantly induces aneuploids (28%), tetraploids (13%) and mitotic arrest in human lymphocytes from four different donors *in vitro* at 0.01 μM,[27] and it also induces tetraploids (22%), which are produced by endoreduplication of DNA, at 10 μM.[28] These results suggest that DMA(III) and arsenite have similar effects on induction of aneuploids and polyploids and on the disturbance of the cell cycle. However, DMA(III) appears to be more active in inducing tetraploidy than arsenite. Additionally, the tetraploids produced by DMA(III) in this experiment were not the result of endoreduplication. Thus, we suggest that trivalent organic arsenic and inorganic arsenic have different effects on the cell cycle.

Many studies observe that arsenite induces chromosomal aberration in cultured cells.[29] Significant increases of chromosomal aberrations were observed in human fibroblasts treated with 3.8 or 7.7 μM sodium arsenite for 24 h, with the main types of aberration observed being chromatid breaks and chromatid gaps.[30] These findings suggest that DMA(III) was at least as clastogenic as arsenite, since DMA(III) significantly induced chromosomal aberrations, most of which were chromatid breaks and chromatid gaps at 2.5 and 5 μM (Table 1).

DMA(III) significantly increased both SCEs per cell and SCEs per chromosome at 0.625 μM (Table 2). Many studies have concluded that arsenite significantly increases SCEs per cell in CHO cells and human lymphocytes.[28] Kochhar *et al.*[28] reported that arsenite and arsenate markedly increased SCEs in CHO cells over the range of 0.01–10 μM. Larramendy *et al.*[31]

observed a significant increase in SCEs in CHO cells and human lymphocytes at an arsenite concentration of 0.1 μM. These findings suggest that the SCE-inducing activity of DMA(III) is as high as arsenite. However, the activity of DMA(III) was still 1000-fold less than that of MMC (Table 2).

DMA(V) gives similar effects on V79 cells as DMA(III) at more than 100-fold high concentrations of DMA(III) such as mitotic arrest, induction of tetraploids[25,32] and chromosomal aberration.[33] Cysteine enhances the cytogenetic toxic effects of DMA(V) in V79 cells to induce mitotic arrest, tetraploid formation and induction of chromosomal aberration,[33] and it also enhances cytotoxicity and induction of apoptosis in HL-60 cells.[34] We detected DMA(III) in a mixture of cysteine and DMA(V), and concluded that enhancement of cytogenetic toxicity was due to formation of DMA(III) from DMA(V). Our results in the present report support their conclusion. DMA(V) inhibits tubulin assembly and the GTPase activity of tubulin[35] *in vitro* and induces the formation of abnormal spindles in mitotic cultured cells.[36] It would be interesting to examine whether or not DMA(III) has the same activity at low concentration.

Our results show that DMA(III) was as genotoxic in causing chromosomal aberrations and SCEs as arsenite. However, DMA(III) was much more active in inducing tetraploids than arsenite, since the rate of induced tetraploids by DMA(III) was about fourfold higher than arsenite. Arsenite is generally found to be non-carinogenic according to standard carcinogenicity bioassays.[37] DMA(III), *per se*, may be more inherently carcinogenic than arsenite, since the induction of aneuploidy and/or polyploidy are considered to be closely related to carcinogenicity.[38,39]

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予想される結果と本研究の意義

国内外で統合医療に関する研究が精力的に進められているが，その科学的な評価法を確立しようとする研究はみあたらない．この研究の特色は漢方専門医と西洋医学専門医(消化器病専門医)が協力して行う患者中心主義医療のなかで，大規模比較試験に基づく統計学では判定困難な統合医療に対して，その有効性を"患者中心主義の医療"のなかで科学的に評価しようとする点に特色があり，東洋医学と西洋医学の融合をめざした新しい臨床研究手法と考えられる．さらに，舌診の画像解析もこれまで行われていなかった方法であり，プロテオームにより発見されるマーカーとの相関解析から過敏性腸症候群の有効性を評価する科学的基準の提案にもつながる意義は大きいと考えられる．

おわりに

過敏性腸症候群患者を対象として漢方医学と西洋医学を融合した統合医療を行い，以下の点を明らかにする．

① 漢方専門医と西洋医学医(消化器病専門医)が協力した患者中心主義の新しい臨床研究の提案．

② "患者による治療有効性の評価"と"東洋医学的診断法のひとつである舌診の画像解析による客観的診断法"の相関．

③ "患者による治療有効性の評価"と"血清を用いた網羅的蛋白質解析(プロテオーム)"の相関解析により統合医療の有効性を評価できる血清バイオマーカーの同定．

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環境衛生

海産物のヒ素
——その健康リスク

Arsenic in marine products——the human risk assessment

イギリス食品規格庁(Food Standards Agency：FSA)は，2004年7月28日にヒジキを食べないようにイギリス国民に対して勧告を出した．その理由はFSAの調査で，ヒジキに発癌リスクの指摘されている無機ヒ素が多く含有していることが判明したためとしている．それに対し厚生労働省は同年7月30日にQ and Aを発表し，「WHOが1988年に定めた無機ヒ素のPTWI(暫定的耐容週間摂取量)は15 μg/kg体重/wkであり，体重50 kgのヒトの場合，107 μg/人/day(750 μg/人/wk)に相当します．FSAが調査した乾燥品を水戻ししたヒジキ中の無機ヒ素濃度は最大で22.7 mg/kgでしたが，かりにこのヒジキを摂食するとしても毎日4.7 g(1週間当り33 g)以上を継続的に摂取しないかぎり，ヒ素のPTWIを超えることはありません．(中略)ヒジキを極端に多く摂取するのではなく，バランスのよい食生活を心がければ健康上のリスクが高まることはないと思われます」とコメントしている．

無機ヒ素は図1のように哺乳動物の体内で還元とメチル化を繰り返して有機化される．海産物には図1に示す有機ヒ素化合物のほか，種々の動植物で合成されたアルセノベタイン(AsBe)やアルセノコリンなどが多く含まれている．

究極発癌物質は？

無機ヒ素を曝露したヒトにおいては，急性中毒，慢性中毒，さらには癌の発症が認められている．また，有機化されるにつれ急性毒性が弱くなるので，代謝は解毒と考えられ，ヒ素の毒性として無機ヒ素が注目されていた．しかし，その後，発癌性については無機ヒ素の主要代謝物であるジメチルアルシン酸〔DMA(V)〕が *in vitro*, *in vivo* で4倍体形成や染色体異常を起こし，動物実験で癌を誘発したことからDMA(V)が究極発癌物質ではないかと疑われるようになった．さらに，現在ではその還元型であるDMA(Ⅲ)またはその近傍の化合物に焦点が移っている．

究極発癌物質がDMA(Ⅲ)またはその近傍であるとするならば，発癌リスクアセスメントに用いる曝露量は無機ヒ素からDMA(Ⅲ)

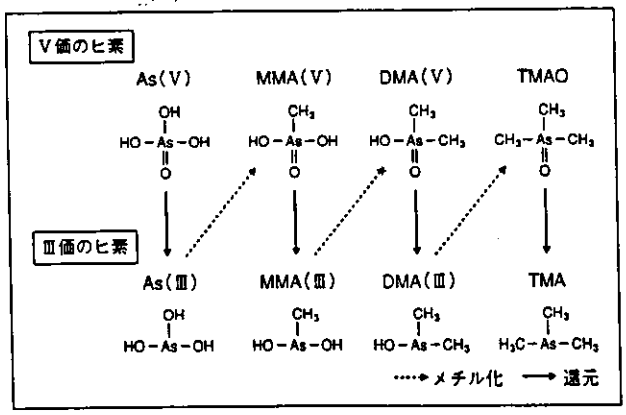

図1 ヒ素の代謝経路

までの総和が対象となる．Ⅲ価のヒ素化合物は不安定で，あまり検出されない．そのため，アメリカ労働衛生専門家会議(ACGIH)の生物学的曝露指標値(biological exposure indices：BEIs)では職業性に無機ヒ素を曝露する労働者に対し1週間の作業終了時に採尿し，ヒ酸，亜ヒ酸とメチルアルソン酸[MMA(V)]，DMA(V)の合計が35 μgAs/l 以下とするよう勧告している．

著者らの検討結果

表1のAは，海産物を多く摂取したときの著者(圓藤吟史)の尿である．教室員9名で，海産物を多く摂取してから測ったところ総ヒ素量は著者が一番多く検出された．内訳はAsBeがもっとも多く，ついでDMA(V)であった．海産物を摂取しないようにするときわめて低い値になる．著者の尿からは無機ヒ素とMMA(V)は検出されなかったが，教室員8名でのそれらの合計は0～10 μgAs/l の範囲で検出された．海産物に多量に含まれるAsBeは毒性がきわめて弱く，また哺乳動物の体内では代謝されずそのまま尿から排泄されることから，発癌リスクの計算から除外される．海産物を摂取せず野菜食を1カ月続けたBではすべてのヒ素化合物がきわめて低い値になった．Cは，バングラディシュの高濃度の無機ヒ素が含まれる井戸水(600 μgAs/l)を飲用して総ヒ素量がもっとも多く検出されたヒ素中毒者の尿の測定結果である．この地方の人は海産物を摂取しないので，AsBeは検出されない．この患者の尿は無機ヒ素からDMA(V)までの和が1,264 μgAs/l となりハイリスクと考えられる．

表1　尿中濃度の例　　単位(μgAs/l)

	As (V)	As (Ⅲ)	MMA (V)	DMA (V)	TMAO	TeMA	AsBe	unkn-own	total
A	N. D.	N. D.	N. D.	58	N. D.	N. D.	425	8	491
B	N. D.	1.1	0.8	6.2	N. D.	N. D.	3.5	N. D.	11.6
C	25	223	371	644	N. D.	N. D.	N. D.	N. D.	1,264

As(V)：ヒ酸，As(Ⅲ)：亜ヒ酸，MMA(V)：メチルアルソン酸，DMA(V)：ジメチルアルシン酸，TMAO：トリメチルアルシンオキシド，TeMA：テトラメチルアルソニウム，AsBe：アルセノベタイン，unknown：未知のヒ素化合物，N. D.：検出限界 0.1 μgAs/l 以下

おわりに

Aのように海産物を多く摂取する者はしばしば尿中濃度が35 μgAs/l を超える．いままで海産物に含まれるヒ素により健康被害が起こったとの報告はないが，発癌性のリスクを考えるならば，今後ヒジキに含まれる無機ヒ素のみならず，MMA(Ⅲ)，DMA(V)も含めて耐容摂取量を決めていかなければならない．

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