Trace Nutrients Research 21:65-72
(2004)

Studies on the Mechanisms of Arsenic Tolerance Induced in Rat Liver Cells by Chronic Exposure to Methylated Arsenicals

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Summary

In this study, we examined the effects of chronic low-level exposure to methylated arsenicals on tolerance to inorganic arsenite *in vitro* using the normal rat liver cell line TRL 1215. For 20 weeks, cells were exposed to methylated mammalian metabolites of inorganic arsenicals; monomethylarsonic acid ($MMAs^V$; 1.3 mM), dimethylarsinic acid ($DMAs^V$; 700 μM) or trimethylarsine oxide ($TMAs^VO$; 10 mM). These cells acquired tolerance to the acute cytolethality of inorganic arsenite. Cellular arsenic accumulation was less in chronic methylated arsenical-exposed cells than passage-matched control cells. Long-term exposure to methylated arsenicals increased cellular glutathione *S*-transferase (GST) activity and cellular reduced glutathione (GSH) levels, although only chronic exposure to $DMAs^V$ significantly decreased cellular GSH levels. Cellular GSH depletion increased arsenic accumulation and decreased tolerance to the acute cytolethality of arsenite in these chronic methylated arsenical-exposed cells. These results indicate that chronic exposure to methylated arsenicals induces tolerance to inorganic arsenite, and that cellular GSH may play an important role in the induction of this tolerance.

Introduction

Arsenic is a metalloid and is widely distributed in the water, soil and air. Arsenic has been notorious for its toxicity, and it has been reported that arsenic poisoning has occurred in some countries in Asia and the Americas through the consumption of contaminated well water or food[1, 2]. In the environment, arsenic exists as its inorganic form, mainly the pentavalent form (arsenate; As^V) and sometimes as its trivalent form (arsenite; As^{III})[1, 2]. Pentavalent arsenate is easily reduced to trivalent arsenite[3], which is then enzymatically methylated in humans and many experimental animals. A study has shown that monomethylarsonic acid ($MMAs^V$) and dimethylarsinic acid ($DMAs^V$) are the major pentavalent organic metabolites in human urine after the exposure to inorganic arsenicals[4]. $DMAs^V$ is the ultimate metabolite in humans, while $DMAs^V$ is further methylated to trimethylarsine oxide ($TMAs^VO$) in some rodents (Fig. 1)[5].

Epidemiological studies provide clear evidence that inorganic arsenicals are a carcinogen to humans, involving the liver, skin, lung, kidney, and urinary bladder as target sites[1, 2]. Inorganic arsenicals also have pronounced acute toxicity in humans and experimental animals[2]. Because animal models for inorganic arsenic toxicity are limited, and in some cases, equivocal, *in vitro* systems have been widely used to define the molecular events associated with inorganic arsenic carcinogenicity and toxicity. This approach has provided useful information on the genotoxic effects of inorganic arsenicals, on the inorganic arsenicals-induced mutations, on the inorganic arsenicals-induced changes in the methylation status of DNA, and on the inorganic arsenicals-induced expression of genes involved in the regula-

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tion of cellular growth and proliferation[6, 7]. In contrast, there has been little information on the detailed evaluation and the mechanisms of the toxic effects of methylated arsenicals as the metabolites of inorganic arsenicals in mammals.

Originally, the methylation of inorganic arsenials was accepted to be a detoxification system due to the minimizing of the acute toxicity as indicated by their increase in the 50 % lethal doses (LD_{50}s) *in vivo*[8] and the 50 % lethal concentrations (LC_{50}s) *in vitro*[9]. However, recent evidence increasingly suggests that the methylation of inorganic arsenicals is not a universally detoxifying event, because it has been often reported that $DMAs^V$ has a significant genotoxicity or carcinogenicity *in vitro* and *in vivo*[10, 11]. We also recently reported that $DMAs^V$ primarily induces apoptosis in mammalian cells *in vitro* and requires cellular reduced glutathione (GSH) to become cytotoxic[12-15]. It has been reported that methylated arsenicals accumulate during chronic arsenic poisoning in human body[16]. On the other hand, arsenic has been used as therapeutic agents for many years. Recently, inorganic arsenite was shown to be effective in inducing complete remission in patients with acute promyelocytic leukemia (APL)[17]. Clinically, multiple high doses of inorganic arsenite by intravenous injection (10 mg/day) for at least 28 consecutive days are needed to induce complete remission in APL patients[17], so the accumulation of the methylated forms of arsenic may occur in the patients. It would appear that defining the mechanisms of chronic arsenic poisoning, and perhaps carcinogenesis, requires further studies on the effects of the chronic exposure to methylated arsenicals. However, the details on the effects of chronic exposure to $DMAs^V$ has not yet been clarified, and much less is known concerning the *in vitro* toxic potential or mechanisms of the other pentavalent methylated arsenic metabolites, $MMAs^V$ and $TMAs^VO$.

We demonstrated in this study that chronic exposure to low-levels of $MMAs^V$, $DMAs^V$ and $TMAs^VO$ induced tolerance to the acute cytolethality of inorganic arsenite using rat liver cells. The liver is a major site of arsenic methylation and accumulation, and is also a target tissue of arsenic toxicity and carcinogenicity.

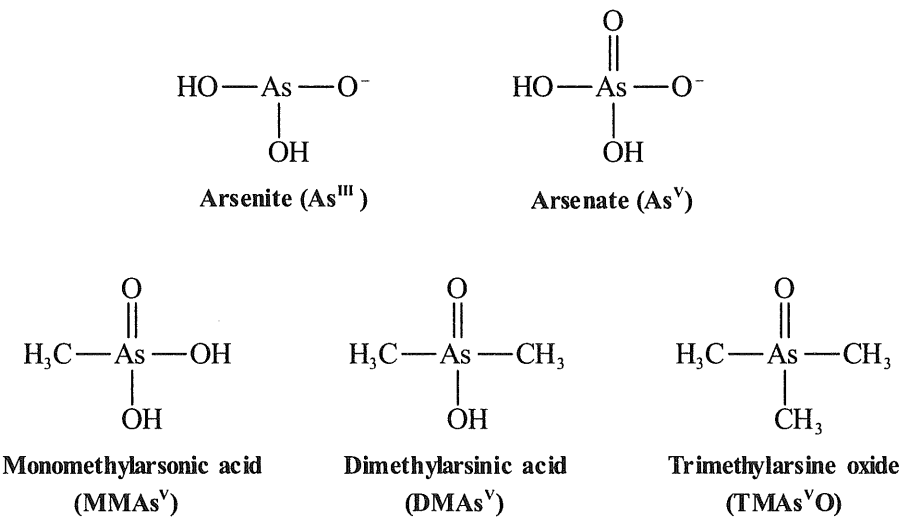

Fig. 1 Primary structure of inorganic and methylated arsenicals

Materials and methods

Chemicals. Monomethylarsonic acid ($MMAs^V$) was obtained from Tri Chemical Laboratory Inc. (Yamanashi, Japan). Sodium arsenite and dimethylarsinic acid ($DMAs^V$) were purchased from Wako Pure Chemical Co. (Osaka, Japan). Trimethylarsine oxide ($TMAs^VO$) was synthesized from trimethylarsine using hydrogen peroxide as

described elsewhere[18]. These arsenicals were recrystallized twice and their purities were > 99.9 % as determined by gas chromatography-mass spectrometry[12]. Endotoxin contamination of these arsenicals was < 0.0000003 % (wt/wt) as determined by the endotoxin-specific limulus test (Seikagaku Co., Tokyo, Japan). Reduced glutathione (GSH) and L-buthionine-(*S,R*)-sulfoximine (BSO; an inhibitor of γ-glutamylcysteine synthetase which decreases cellular GSH levels) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Cell culture condition. TRL 1215 cell line was generously provided by Dr. Michael P. Waalkes (National Cancer Institute at National Institute of Environmental Health Sciences, National Institute of Health, NC, USA). TRL 1215 cells are adhesive rat epithelial liver cells originally derived from the liver of 10-day old Fisher F344 rats[6] and were cultured in William' s medium E (Sigma) supplemented with 10 % fetal bovine serum, 2 mM glutamine and antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin) under a humidified atmosphere of 5 % CO_2/95 % air at 37℃.

Chronic arsenic exposure. TRL 1215 cells were chronically exposed to $MMAs^V$ (1.3 mM), $DMAs^V$ (700 μM) or $TMAs^VO$ (10 mM) for 20 weeks in the 25 cm^2 tissue culture flasks. These concentrations were 1/10 of acute cytotoxic concentrations (LC_{50} values for 48 h *in vitro* incubation) of these methylated arsenicals in TRL 1215 cells[12-15]. Control cells were incubated with medium alone for 20 weeks.

Assay for acute arsenic cytolethality. Control cells and chronic methylated arsenical-exposed cells were isolated by trypsinization, washed twice and resuspended in fresh medium. Samples of 1×10^4 cells/100 μl/well were plated in flat-bottomed 96-well tissue culture plates and allowed to adhere to the plate for 24 h at which time the medium was removed and replaced with fresh medium containing inorganic arsenite. Cells were then incubated with arsenite for an additional 48 h. After the incubation, cells were washed twice with warmed phosphate-buffered saline (pH = 7.4) to remove non-adherent dead cells, and cellular viability was determined by AlamarBlue assay, which is similar to MTT assay and measured metabolic integrity[12]. Briefly, after the incubation with arsenite and replacement with 100 μl fresh media, 10 μl/well AlamarBlue solution (Iwaki Grass Co., Chiba, Japan) was added directly to the wells, incubated at 37℃ for 4 h, and the absorbance at 570 nm (reference as 600 nm) was measured by a microplate reader model 550 (Bio-Rad Laboratories, Hercules, CA, USA). Data are expressed as relative metabolic integrity using the values from control cells as 100 %.

Arsenic analysis. Control cells and chronic methylated arsenical-exposed cells that were grown in flat-bottomed 75 cm^2 tissue culture flask (8×10^7 cells/flask) were pre-incubated with or without 25 μM BSO for 24 h, and were further exposed to 5 μM arsenite for an additional 48 h. After the exposure, the amounts of cellular arsenic in these cells were analyzed by hydride generation coupled with atomic absorption spectrometry (AAS) using SpestraAA-220 (Varian Australia Pty Ltd., Mulgrave, Victria, Australia)[19]. The results are expressed as ng of the cellular arsenic contents per mg of cellular protein determined by BCA protein assay (Pierce Co., Rockford, IL, USA) with bovine serum albumin as a standard, or the cellular arsenic concentrations calculated using the total cell numbers and the estimated cell density (2.4×10^{-9} cm^3/cell) with hemacytometer counting chamber[20].

Assay for cellular glutathione S-transferase (GST) activity. Cellular GST activity was measured by the method of Lee *et al.* (1989) using 1-chloro-2,4-dinitrobenzene and GSH as substrates[21]. Cells were isolated by trypsinization, rinsed twice with phosphate-buffered saline (pH = 7.4), resuspended in 400 μl of 100 mM potassium phosphate buffer (pH = 6.8), and sonicated for 10 s on ice. Cellular debris was removed by centrifugation, and 50 μl of the supernatant of each cell was then mixed with 850 μl of 100 mM sodium phosphate buffer including 1 mM ethylenediamine tetraacetate (EDTA, pH = 6.5), 50 μl of 20 mM GSH and 50 μl of 20 mM 1-chloro-2,4-dinitrobenzene at room temperature, and the absorbance at 340 nm was continuously measured for 2 min. Data are expressed as specific GST activity (nmol/min) per mg of cellular protein determined by BCA protein assay.

Assay for cellular reduced glutathione (GSH) levels. Cellular GSH levels were measured by the method of Hissin and Hilf (1976) using *o*-phthaldialdehyde as a substrate[22]. Cells were isolated by trypsinization, rinsed twice with phosphate-buffered saline (pH = 7.4), and digested in 150 μl of ice cold 800 mM perchloric acid including 8 mM EDTA. Cellular debris was removed by centrifugation, and 100 μl of the supernatant of each cell was then added into 2 ml of 100 mM sodium phosphate buffer (pH = 8.0) including 5 mM EDTA and 1 mg/ml *o*-phthaldialdehyde, and was kept for 15 min at room temperature in the dark. The fluorescence intensity of the sample solutions was measured at excitation and emission wavelengths of 350 nm and 425 nm, respectively. Aliquots of GSH were used to construct a standard curve, and the results are expressed as GSH nmol per mg of cellular protein determined by BCA protein assay.

Statistics. Statistical evaluations in experiments were expressed as the arithmetic mean ± SEM and performed by ANOVA followed by Dunnett's multiple comparison test or the Student's *t*-test as appropriate. A value of $p < 0.05$ was considered significant in all cases.

Results

Chronic Methylated Arsenic Exposure Induced Tolerance to Acute Inorganic Arsenite Cytolethality in Rat Liver TRL 1215 Cells

TRL 1215 cells were incubated with $MMAs^V$ (1.3 mM), $DMAs^V$ (700 μM), $TMAs^VO$ (10 mM) or medium alone (control) for 20 weeks. These concentrations of methylated arsenicals had no effect on viability of TRL 1215 cells after 20 weeks incubation. The cellular arsenic contents in these chronic methylated arsenical-exposed cells were 135.4 ± 12.0 ng/mg cellular protein (121.8 ± 10.8 nM), 41.8 ± 2.5 ng/mg (37.6 ± 2.3 nM) or 543.8 ± 12.2 ng/mg (489.4 ± 11.0 nM) when cells were chronically exposed to $MMAs^V$, $DMAs^V$ and $TMAs^VO$, respectively. These chronic methylated arsenical-exposed cells were further exposed to various concentrations of inorganic arsenite for an additional 48 h, and cell viability was then assessed by AlmarBlue assay. As shown in Fig. 2, these chronic methylated arsenical-exposed cells acquired tolerance to the acute cytolethality of arsenite. The LC_{50} values for acute arsenite exposure were as follows: chronic $MMAs^V$-exposed cells (75.5 μM) > chronic $TMAs^VO$-exposed cells (67.5 μM) >> chronic $DMAs^V$-exposed cells (19.2 μM) > control cells (18.4 μM).

We previously reported that cellular GSH depletion enhanced the cytolethality of arsenite[12-15]. We subsequently observed the effects of cellular GSH depletion by BSO treatment on the arsenic tolerance induced by chronic exposure to methylated arsenicals. BSO (25 μM) treatment significantly decreased the tolerance to acute arsenite cytolethality in chronic methylated arsenical-exposed cells (Fig. 2).

Changes in Cellular GST Activity and GSH Levels by Chronic Methylated Arsenic Exposure in Rat Liver TRL 1215 Cells

Cellular GST activity and GSH levels in chronic methylated arsenical-exposed cells were measured. As shown in Fig. 3, chronic exposure to $MMAs^V$, $DMAs^V$ or $TMAs^VO$ significantly increased cellular GST activity in TRL 1215 cells. Figure 4 shows the effects of chronic exposure of methylated arsenicals on cellular GSH levels in TRL 1215 cells. $MMAs^V$ and $TMAs^VO$ markedly increased cellular GSH levels, but chronic $DMAs^V$ exposure decreased them.

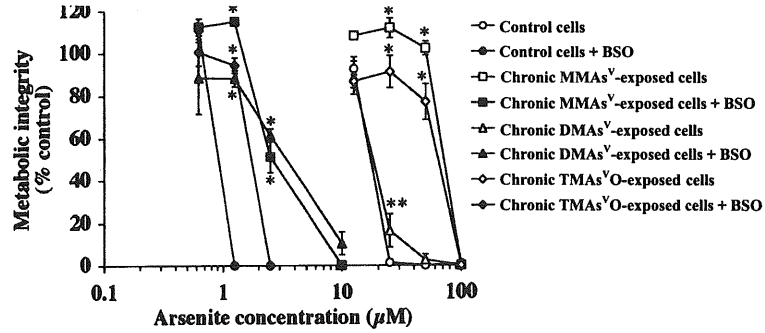

Fig. 2 Acute *in vitro* cytolethality of arsenite in chronic methylated arsenical-exposed cells. TRL 1215 cells were incubated with $MMAs^V$ (1.3 mM), $DMAs^V$ (700 μM), $TMAs^VO$ (10 mM) or medium alone (control) for 20 weeks. After the incubation, these cells were incubated with or without 25 μM BSO for 24 h, and were further exposed to various concentrations of arsenite in the presence or absence of BSO for an additional 48 h. Cell viability was then assessed. Results are expressed as arithmetic mean ± SEM of three separate experiments performed in triplicate (n = 9). *p < 0.001 comparison with control cells, **p < 0.05.

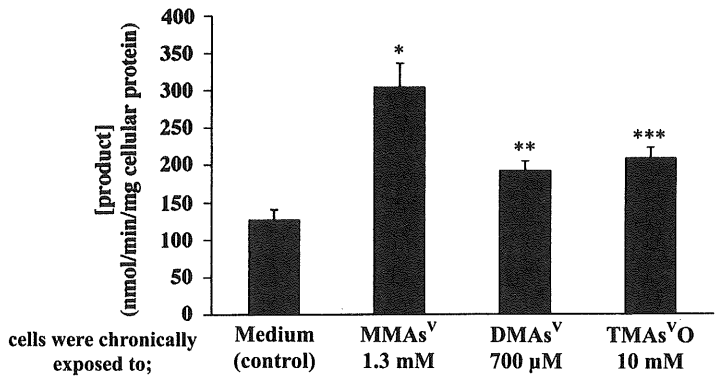

Fig. 3 Effect of chronic exposure to methylated arsenicals on cellular GST activity in TRL 1215 cells. Cells were incubated with $MMAs^V$ (1.3 mM), $DMAs^V$ (700 μM), $TMAs^VO$ (10 mM) or medium alone (control) for 20 weeks, and cellular GST activity were then measured. Results are expressed as arithmetic mean ± SEM of three separate experiments performed in triplicate (n = 9). *p < 0.001 comparison with control cells, **p < 0.01, ***p < 0.05.

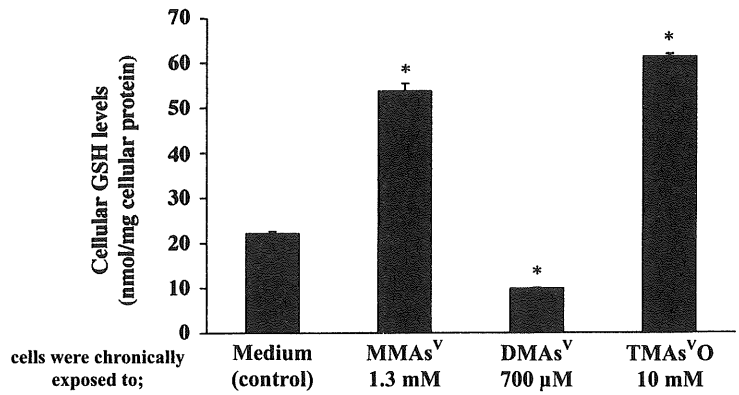

Fig. 4 Effect of chronic exposure to methylated arsenicals on cellular GSH levels in TRL 1215 cells. Cells were incubated with $MMAs^V$ (1.3 mM), $DMAs^V$ (700 μM), $TMAs^VO$ (10 mM) or medium alone (control) for 20 weeks, and cellular GSH levels were then measured. Results are expressed as arithmetic mean ± SEM of three separate experiments performed in triplicate (n = 9). *p < 0.001 comparison with control cells.

Chronic Methylated Arsenic Exposure Decreased Cellular Arsenic Accumulation in Rat Liver TRL 1215 Cells

Chronic methylated arsenical-exposed cells were further incubated with 5 μM inorganic arsenite for 48 h, and cellular arsenic contents of these cells were then measured by AAS. As shown in Table 1, chronic exposure to $MMAs^V$, $DMAs^V$ or $TMAs^VO$ significantly decreased the cellular arsenic accumulation in TRL 1215 cells. When cellular GSH was depleted by BSO treatment, cellular arsenic accumulation in these chronic methylated arsenical-exposed cells was significantly augmented.

Table 1 Cellular arsenic accumulation in chronic methylated arsenical-exposed cells

cells were chronically exposed to;	Cellular arsenic contents (ng/mg cellular protein) −	+ BSO
Medium (control)	59.1 ± 13.9	235.5 ± 31.0 †
$MMAs^V$ 1.3 mM	19.5 ± 1.5 *	94.4 ± 21.1** ††
$DMAs^V$ 700 μM	19.1 ± 1.6 *	72.8 ± 6.0*** †
$TMAs^VO$ 10 mM	26.1 ± 1.4 *	147.1 ± 7.0 * †

TRL 1215 cells were incubated with $MMAs^V$ (1.3 mM), $DMAs^V$ (700 μM), $TMAs^VO$ (10 mM) or medium alone (control) for 20 weeks. After the incubation, these cells were washed and incubated with or without 25 μM BSO for 24 h, and were further exposed to 5 μM arsenite for an additional 48 h. The cellular arsenic contents were then measured by AAS. Results are expressed as arithmetic mean ± SEM of three separate experiments performed in triplicate (n = 9).
* $p < 0.05$ comparison with control cells, ** $p < 0.01$, *** $p < 0.001$.
† $p < 0.001$ comparison with the cells which were incubated with same arsenicals or medium alone for 20 weeks and further exposed to arsenite without BSO, †† $p < 0.01$.

Discussion

The present study demonstrates that tolerance to the acute cytolethality of arsenite is induced in mammalian cells by chronic exposure to non-toxic levels of $MMAs^V$, $DMAs^V$ and $TMAs^VO$. Rat liver TRL 1215 cells were exposed to micro- to millimolar levels of methylated arsenicals for 20 weeks, and the cellular arsenic contents of these cells after exposure was in the nanomolar range. We thus observed the effects of chronic exposure to nanomolar cellular levels of methylated arsenicals on arsenic sensitivity in rat liver cells. Methylated arsenicals are formed from inorganic arsenicals by enzymatic methylation in mammalian cells[23], and it has been reported that urinary concentrations of methylated arsenicals in chronic arsenic poisoning patients in Inner Mongolia, China were in the micromolar range[16]. This suggests that the use of nanomolar cellular arsenic concentrations for *in vitro* experiments may, at least a part, reflect *in vivo* conditions.

Chronic methylated arsenic exposure induced significant tolerance to the acute toxicity of inorganic arsenite. Because chronic arsenical-exposed cells had an enhanced ability to excrete cellular arsenic, the arsenic tolerance induced by chronic exposure to methylated arsenicals appears to be based on increased arsenic efflux. Cellular GSH may also play a critical role in this chronic methylated arsenical-exposure induced arsenic tolerance. GSH may decrease the cytolethality of arsenic through several processes, possibly through its role as an antioxidant, through its role as a co-factor in the enzymatic methylation reaction of arsenic, by directly binding arsenic and thereby reducing the toxic potential, or through enhanced efflux of an arsenic-GSH conjugation[9]. It has been reported that arsenic-GSH conjugates are excreted via some cell membrane transporters[6, 24]. In this study, chronic exposure to $MMAs^V$ and $TMAs^VO$ increased cellular GST activity and cellular GSH levels. GST catalyzes the formation of arsenic-GSH conjugates[6, 24]. Taken together, these data suggest that chronic exposure to $MMAs^V$ and $TMAs^VO$ might induce a cellular GSH-dependent arsenic excretion mechanism.

Chronic exposure to $DMAs^V$ also increased cellular GST activity, but decreased cellular GSH levels. Chronic $DMAs^V$-exposed cells maintained their arsenic tolerance to arsenite (Fig. 1) and the ability to excrete cellular arsenic (Table 1), even when cellular GSH was depleted. Long-term exposure to $DMAs^V$ would induce not only cellular GSH-dependent arsenic excretion pathways but also other arsenic excretion pathways using specific cell membrane transporters, such as P-glycoprotein[25]. Further research is required in order to clarify the molecular mechanisms of the induction of arsenic tolerance by chronic exposure to $DMAs^V$ in mammalian cells.

In conclusion, the present study indicates that chronic exposure to $MMAs^V$, $DMAs^V$ and $TMAs^VO$ induces tolerance to acute inorganic arsenic cytolethality in rat liver cells. Chronic exposure to methylated arsenicals appears to induce GSH-dependent arsenic excretion pathways and other arsenic excretion pathways. This study may have implications in treating chronic arsenic poisoning and in the long-term chemotherapeutic use of arsenic.

Acknowledgements We express our thanks to Mr. Takeshi Gomyou, Mr. Ayumu Mizukami, and Miss Tomomi Suzuki for their excellent technical assistance.

References

1) IARC (1987) International Agency for Research on Cancer Monographs on the Evaluation of the Carcinogenic Risks to Humans: Supplement 7, Overall evaluations carcinogenicity: An updating of IARC monographs, IARC Scientific Publications, Lyon, France: Vol. 1-42, pp 100-106.
2) NRC (1999) Arsenic in the Drinking Water, National Research Council, National Academy Press, Washington, DC.
3) Vahter M, Envall J (1983) *In vivo* reduction of arsenate in mice and rabbits. Environ Res 32: 14-24.
4) Yamauchi H, 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.
5) Yamauchi H, Yamamura Y (1984) Metabolism and excretion of orally administered dimethylarsinic acid in the hamster. Toxicol Appl Pharmacol 74: 134-140.
6) Chen H, Liu J, Merrick BA, Waalkes MP (2001) Genetic events associated with arsenic-induced malignant transformation: applications of cDNA microarray technology. Mol Carcinog 30: 79-87.
7) Zhao CQ, Young MR, Diwan BA, Coogan TP, Waalkes, MP (1997) Association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expression. Proc Natl Acad Sci USA 94: 10907-10912.
8) Kaise T, Yamauchi H, Horiguchi Y, Tani T, Watanabe S, Hirayama T, Fukui S (1989) A comparative study on acute toxicity of methylarsonic acid, dimethylarsinic acid and trimethylarsine oxide in mice. Appl Organomet Chem 3: 273-277.
9) Romach EH, Zhao CQ, Del Razo LM, Cebrian ME, Waalkes MP (2000) Studies on the mechanisms of arsenic-induced self tolerance developed in liver epithelial cells through continuous low-level arsenite exposure. Toxicol Sci 54: 500-508.
10) Tezuka M, Hanioka K, Yamanaka K, Okada S (1993) Gene damage induced in human alveolar type II (L-132) cells by exposure to dimethylarsinic acid. Biochem Biophys Res Commun 191: 1178-1183.
11) Wei M, Wanibuchi H, Morimura K, Iwai S, Yoshida K, Endo G, Nakae D, Fukushima S (2002) Carcinogenicity of dimethylarsinic acid in male F344 rats and genetic alterations in induced urinary bladder tumors. Carcinogenesis 23: 1387-1397.
12) Sakurai T, Qu W, Sakurai MH, Waalkes MP (2002) A major human arsenic metabolite, dimethylarsinic acid,

requires reduced glutathione to induce apoptosis. Chem Res Toxicol 15: 629 - 637.

13) Sakurai T (2002) Molecular mechanisms of dimethylarsinic acid-induced apoptosis. Biomed Res Trace Elements 13: 167 - 176.

14) Sakurai T (2003) Biomethylation of arsenic is essentially detoxicating event. J Health Sci 49: 171 - 178.

15) Sakurai T, Kojima C, Ochiai M, Ohta T, Sakurai MH, Waalkes MP, Fujiwara K (2004) Cellular glutathione prevents cytolethality of monomethylarsonic acid. Toxicol Appl Pharmacol 195: 129 - 141.

16) Yamauchi H (2000) Metabolism of arsenic in the Mammalian: mainly the case with the acute arsenic poisoning. Biomed Res Trace Elements 11: 25 - 34.

17) Shen ZX, Chen GQ, Ni JH, Li XS, Xiong SM, Qiu QY, Zhu J, Tang W, Sun GL, Yang KQ, Chen Y, Zhou L, Fang ZW, Wang YT, Ma J, Zhang P, Zhang TD, Chen SJ, Chen Z, Wang ZY (1997) Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. Blood 89: 3354 - 3360.

18) Kaise T, Hanaoka K, Tagawa S (1987) The formation of trimethylarsine oxide from arsenobetaine by biodegradation with marine microorganisms. Chemosphere 16: 2551 - 2558.

19) Ohta T, Sakurai T, Fujiwara K (2004) Effects of arsenobetaine, a major organic arsenic compound in seafood, on the maturation and functions of human peripheral blood monocytes, macrophages and dendritic cells. Appl Organomet Chem 18: 431 - 437.

20) Mizoguchi H, Hara S (1996) Effect of fatty acid saturation in membrane lipid bilayers on simple diffusion in the presence of ethanol at high concentration. J Ferment Bioeng 81: 406 - 411.

21) Lee TC, Wei ML, Chang WJ, Ho IC, Lo JF, Jan KY, Huang H (1989) Elevation of glutathione levels and glutathione *S*-transferase activity in arsenic-resistant Chinese hamster ovary cells. In Vitro Cell Dev Biol 25: 442 - 448.

22) Hissin PJ, Hilf R (1976) A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem 74: 214-226.

23) Styblo M, Del Razo LM, LeCluyse EL, Hamilton GA, Wang C, Cullen WR, Thomas DJ (1999) Metabolism of arsenic in primary cultures of human and rat hepatocytes. Chem Res Toxicol 12: 560 - 565.

24) Kala SV, Kala G, Prater CI, Sartorelli AC, Lieberman MW (2004) Formation and urinary excretion of arsenic triglutathione and methylarsenic diglutathione. Chem Res Toxicol 17: 243 - 249.

25) Liu J, Chen H, Miller DS, Saavedra JE, Keefer LK, Johnson DR, Klaassen CD, Waalkes MP (2001) Overexpression of glutathione *S*-transferase II and multidrug resistance transport proteins is associated with acquired tolerance to inorganic arsenic. Mol Pharmacol 60: 302 - 309.