APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2002; **16**: 421–426
Environment, Biology and Toxicology AOC
Published online in Wiley InterScience (www.interscience.wiley.com). DOI:10.1002/aoc.328

Cytotoxicological aspects of the organic arsenic compound arsenobetaine in marine animals†

Chikara Kojima[1], Teruaki Sakurai[1]*, Masayuki Ochiai[1], Hidetoshi Kumata[1], Wei Qu[2], Michael P. Waalkes[2] and Kitao Fujiwara[1]

[1]Laboratory of Environmental Chemistry, School of Life Science, Tokyo University of Pharmacy and Life Science, Horinouchi 1432-1, Hachioji, Tokyo 192-0392, Japan
[2]Inorganic Carcinogenesis Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute at National Institute of Environmental Health Sciences, National Institute of Health, 111 Alexander Drive, PO Box 12233, MD F0-09, Research Triangle Park, NC 27709, USA

Received 25 December 2001; Accepted 20 April 2002

In this study, we investigated the *in vitro* cytotoxicity of arsenobetaine [AsBe; trimethyl (carboxymethyl) arsonium zwitterion], which is a major organic arsenic compound in marine animals, to various mammalian cells, such as mouse macrophage RAW264.7 cells, rat liver TRL1215 cells and human skin TIG-112 cells, using a synthetic pure material. As a result, we demonstrated that the cytotoxicity of AsBe is very weak even at concentrations over 20 mmol dm^{-3} in all these cells. Also, AsBe did not affect various functions of the murine macrophage RAW264.7 cells, *viz* interleukin-1α production, cellular lysosomal enzyme (acid phosphatase) activity and nitric oxide (NO_2^-) secretion. AsBe showed a weak effect on the reduced glutathione (GSH) levels in these cells, and it slightly reduced the cellular GSH levels for a while. These data suggest that AsBe shows no cytotoxicity but has some effects on mammalian cells. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: arsenobetaine; arsenical; arsenic compound; cytotoxicity; macrophages; liver; skin; marine animal; seafood

INTRODUCTION

Many arsenic compounds have been notorious for their toxicity, but the toxicity varies significantly with the chemical species. It has been previously reported that marine animals contain very high concentrations of arsenic, *viz*. 4–80 μg g^{-1}, and these marine animals are ingested daily as seafood in many countries.[1] The limit for arsenic in drinking water in Japan is 10 μg dm^{-3} and if this limit were applied to seafood, as 10 ng g^{-1}, most of the seafood would be deemed unfit for consumption.[2] Thus, this finding has caused great concern with respect to the health of those people who often ingest considerable amounts of seafood. The arsenicals in marine animals are generally in the form of water-soluble organic arsenic compounds, and the trimethyl (carboxymethyl) arsonium zwitterion, namely arsenobetaine (AsBe), is a major organic compound in marine animals.[3] It has been suggested that AsBe is the final metabolite in the arsenic cycle of marine ecosystems, because AsBe is widely found in various species of marine animals. Therefore, it is very important for us to study the toxic effects of AsBe. However, there have been only a few reports on them because few studies using pure AsBe for biological experiments have been undertaken. Kaise *et al.*[4] first investigated the acute toxicity of AsBe using synthesized pure material and found that it has no acute toxicity in murine models even over 10 g kg^{-1} when it was administered orally.

In this study, we examined the *in vitro* cytotoxic effects of AsBe on various kinds of mammalian cells, *viz*. mouse macrophage RAW264.7 cells, rat liver TRL1215 cells and human skin TIG-112 cells.

*Correspondence to: T. Sakurai, Laboratory of Environmental Chemistry, School of Life Science, Tokyo University of Pharmacy and Life Science, Horinouchi 1432-1, Hachioji, Tokyo 192-0392, Japan.
E-mail: sakurai@ls.toyaku.ac.jp
†This paper is based on work presented at the 10th International Symposium on Natural and Industrial Arsenic (JASS-10), Tokyo, 29–30 November 2001.
Contract/grant sponsor: Takeda Science Foundation, Japan.

EXPERIMENTAL

AsBe

AsBe was synthesized from trimethylarsine by reaction with ethyl β-bromo-propionate in an atmosphere of CO_2 (Tri Chemical Laboratory Inc., Yamanashi, Japan),[3] and was

twice recrystallized from acetone containing a trace of methanol.[4] It produced white prismatic crystals, with a melting point at 204 °C, and its structure was confirmed by ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, high-performance liquid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICP-MS) and fast-atom bombardment MS (FAB-MS). The purity of this synthesized AsBe was >99.9% as determined by thin-layer chromatography, HPLC–ICP-MS and gas chromatography–MS (GC–MS). The synthesized AsBe was also treated with Kurimover I, which is a removal agent for endotoxin (Kurita Water Industries Ltd, Tokyo, Japan). Lipopolysaccharide (LPS) contamination of this AsBe was <0.000 02% (w/w) as determined by the endotoxin-specific limulus test.

Cells

RAW264.7 cells, a mouse macrophage cell line, were cultured in RPMI-1640 medium (Sigma Chemical Co., St Louis, MO) containing 10% heat-inactivated fetal calf serum (FCS–RPMI) at 37 °C in a CO_2 incubator. TRL1215 cells, a nontumorigenic rat liver cell line,[5] were cultured in William's Medium E (Sigma) containing 10% FCS (FCS–William's Medium E) at 37 °C in a CO_2 incubator. TIG-112 cells (JCRB0533), a normal human skin fibroblast cell line,[6] were obtained from the Health Science Research Resources Bank (Osaka, Japan), and were cultured in Eagle's minimum essential medium (MEM; Sigma) containing 10% FCS (FCS–MEM) at 37 °C in a CO_2 incubator.

Assay for viability of cells

RAW264.7 cells (2×10^5 cells/well) were incubated with AsBe in flat-bottomed 96-well tissue culture plates for 48 h at 37 °C in a CO_2 incubator using FCS–RPMI in the presence or absence of L-buthionine-(*S,R*)-sulfoximine (BSO; Sigma; 1 mmol dm^{-3}) or recombinant murine interferon γ (rMu IFNγ; Genzyme Co., Boston, MA; 10 U cm^{-3}) and LPS (0111:B4; Sigma; 100 ng cm^{-3}). TRL1215 cells (5×10^4 cells/well) and TIG-112 cells (1×10^4 cells/well) were incubated with AsBe in flat-bottomed 96-well tissue culture plates for 48 h at 37 °C in a CO_2 incubator, using FCS–William's Medium E and FCS–MEM respectively, in the presence or absence of 1 mmol dm^{-3} BSO. The viability of cells was determined by measuring live cells using the AlamarBlue (AB) assay or crystal violet (CV) assay. The AB assay is similar to the MTT assay. Briefly, 6 h before the end of the incubation, 10 µl/well AB solution (Iwaki Glass Co., Chiba, Japan) was added directly to the 96-well plates, and the absorbance at 570 nm (reference as 630 nm) was measured using a model 550 microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). A CV assay determined the quantity of the cells. Briefly, 50 µl/well CV solution [dissolving 0.05% CV reagent (Wako Pure Chemical Co., Osaka, Japan) in 12% formalin, 10% ethanol and 78% distilled water] was added to the remaining cells on 96-well plates, kept for 20 min at room temperature, and rinsed with water. The CV-stained cells were then dissolved by 50 µl/well distilled water solution containing 0.78% sodium dihydrogenphosphate dihydrate and 50% ethanol, and the absorbance at 595 nm (reference as 450 nm) was measured using a model 550 microplate reader.

Assay for NO_2^- production of RAW264.7 cells

The NO_2^- concentrations in RAW264.7 cell culture supernatants were measured by the Griess reaction according to a described microassay.[7] Briefly, an equal volume of Griess reagent was incubated with the supernatants for 10 min at room temperature and the absorbance was measured at 550 nm (reference as 630 nm) using a model 550 microplate reader. The NO_2^- concentrations were determined using $NaNO_2$ as the standard.

Assay for interleukin-1α (IL-1α) production of RAW264.7 cells

RAW264.7 cells (2×10^5 cells/well) were incubated with AsBe in flat-bottomed 96-well tissue culture plates for 48 h at 37 °C in a CO_2 incubator in the presence or absence of rMu IFNγ (10 U cm^{-3}) plus LPS (100 ng cm^{-3}), and the IL-1α concentrations in the culture supernatants were quantified by a double sandwich enzyme-linked immunosorbent assay (ELISA). A 96-well plate was coated with hamster anti-rMu IL-1α monoclonal antibody (Genzyme) in bicarbonate buffer (pH 9.6). Uncoupled binding sites in the wells were blocked with phosphate-buffered saline (PBS) containing 0.25% bovine serum albumin and 0.05% Tween 20. The wells were incubated with 50 µl of samples in duplicate for 40 min at 37 °C and then exposed to rabbit anti-rMu IL-1α serum (Genzyme). The plate was developed using a peroxidase-labeled goat anti-rabbit immunoglobulin G-Fc fragment (Organon Teknika, West Chester, PA) and peroxidase substrate (TMB microwell peroxidase substrate system, Kirkegadd & Perry Lab., Gaithersburg, MD). Aliquots of rMu IL-1α (Genzyme) were used to construct a standard curve, and the results were calculated as amounts of IL-1α per microgram of cellular protein determined by BCA protein assay reagent (Piece Co., Rockford, IL) with bovine serum albumin as the standard.

Assay for enzyme activity of RAW264.7 cells

RAW264.7 cells (2×10^5 cells/well) were incubated with AsBe in flat-bottomed 96-well tissue culture plates for 48 h at 37 °C in a CO_2 incubator using FCS–RPMI in the presence or absence of rMu IFNγ (10 U cm^{-3}) plus LPS (100 ng cm^{-3}). The cellular lysosomal acid phosphatase activity in the cell lysates (cells were dissolved by 0.1% Triton X-100) was assayed by a previously described method using *p*-nitrophenyl phosphate as the substrate.[8] Enzyme activity was expressed as the amount of *p*-nitrophenol (PNP) per microgram of cellular protein.

Measurement of cellular reduced glutathione (GSH) levels

RAW264.7 cells, TRL1215 cells and TIG-112 cells were incubated with various concentrations and exposure times of AsBe in tissue culture flasks at 37 °C in a CO_2 incubator. The cellular GSH levels were measured by a method described elsewhere using *o*-phthaldialdehyde as the substrate.[9] Briefly, these cells were lysed in 150 µl of ice-cold 0.8 mol dm^{-3} perchloric acid including 8 mmol dm^{-3} EDTA. Cellular debris was removed by centrifugation, and 100 µl of the supernatants of each cell were then added to 2 ml of 0.1 mol dm^{-3} sodium phosphate buffer (pH = 8.0) including 5 mmol dm^{-3} EDTA and 1 mg cm^{-3} *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 per milligram of cellular protein.

Statistics

Statistical evaluations in experiments were expressed as the arithmetic mean plus/minus the standard error of the mean (SEM) and one-way analysis of variance (ANOVA) followed by a *post hoc* Scheffe's *F*-test. Probabilities less than 5% ($P < 0.05$) were considered significant.

RESULTS

Effect of AsBe on the viability and the functions of mouse macrophage RAW264.7 cells *in vitro*

Macrophages are known to be very sensitive to changes in environmental conditions and release various immune inflammatory factors when they are stimulated. Mouse macrophage RAW264.7 cells were incubated with various concentrations of AsBe for 48 h at 37 °C in a CO_2 incubator in the presence or absence of BSO (1 mmol dm^{-3}), which is a selective inhibitor of γ-glutamylcysteine synthetase, the rate-limiting enzyme in the synthesis of GSH, and the viability of the cells was investigated by AB assay or CV assay. AsBe had no cytotoxicity, even at concentrations over 20 mmol dm^{-3}, with or without BSO as examined by the AB assay (Fig. 1). The same results were obtained by the CV assay (data not shown).

RAW264.7 cells were incubated with various concentrations of AsBe for 48 h at 37 °C in a CO_2 incubator in the presence or absence of rMu IFNγ (10 U cm^{-3}) plus LPS (100 ng cm^{-3}), which are known to be potent enhancers of the secretory functions of macrophages, and NO_2^- production from RAW264.7 cells was measured by the Griess reaction according to a previously described microassay.[7] As shown in Fig. 2, NO_2^- production was slightly inhibited by the addition of 20 mmol dm^{-3} AsBe in the presence of rMu IFNγ plus LPS.

RAW264.7 cells were incubated with various doses of AsBe for 48 h at 37 °C in the presence or absence of rMu IFNγ (10 U cm^{-3}) plus LPS (100 ng cm^{-3}), and the concentrations of IL-1α in the culture supernatants were measured by double sandwich ELISA. As shown in Fig. 3, AsBe had no effect on the release of IL-1α from the RAW264.7 cells.

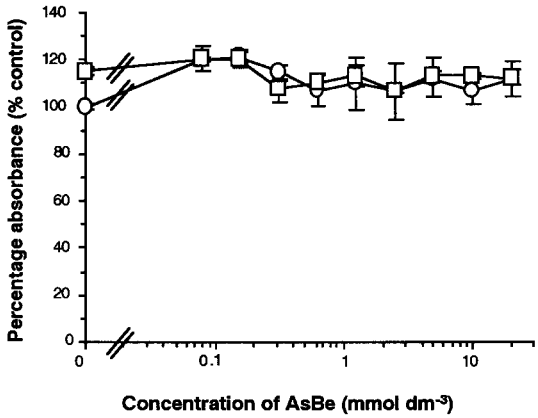

Figure 1. Cytotoxicity of AsBe in mouse macrophage RAW264.7 cells. The RAW264.7 cells were incubated with AsBe in the presence (□) or absence (○) of 1 mmol dm^{-3} BSO for 48 h at 37 °C, and the viability of the cells was determined by the AB assay. One representative experiment out of three similar ones is shown. The results are expressed as the arithmetic mean plus/minus SEM of triplicate dishes.

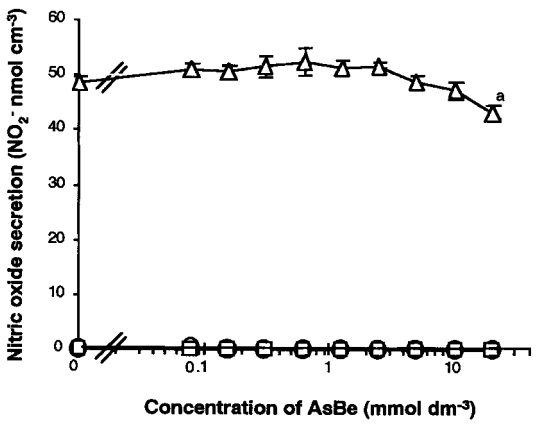

Figure 2. Effect of AsBe on nitric oxide (NO_2^-) production in RAW264.7 cells. The RAW264.7 cells were incubated with AsBe in the presence of 1 mmol dm^{-3} BSO (□) or 10 U cm^{-3} rMu IFNγ plus 100 ng cm^{-3} LPS (△) or medium alone (○) for 48 h at 37 °C, and the NO_2^- concentrations in the culture supernatants were measured as noted in the Experimental section. One representative experiment out of three similar ones is shown. The results are expressed as arithmetic mean plus/minus SEM of triplicate dishes. [a] $P < 0.05$ comparison with RAW264.7 cells incubated with medium alone.

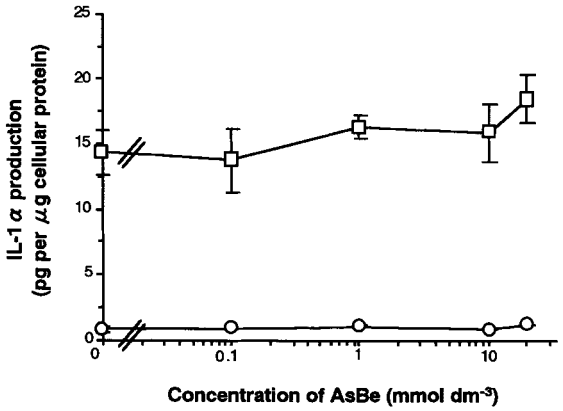

Figure 3. Effect of AsBe on IL-1α production in RAW264.7 cells. The RAW264.7 cells were incubated with AsBe in the presence of 10 U cm^{-3} rMu IFNγ plus 100 ng cm^{-3} LPS (□) or medium alone (○) for 48 h at 37 °C, and the IL-1α productions in the culture supernatants were measured by ELISA. The results are expressed as arithmetic mean plus/minus SEM of duplicate dishes.

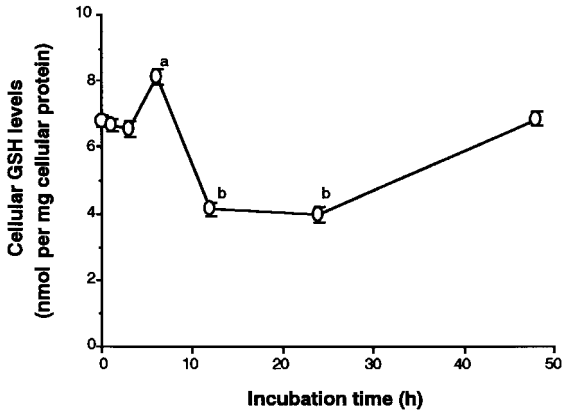

Figure 5. Effect of AsBe on the cellular GSH levels of RAW264.7 cells. The RAW264.7 cells were incubated for various exposure times with 10 mmol dm^{-3} AsBe at 37 °C, and the cellular GSH levels were measured by a method described elsewhere using *o*-phthaldialdehyde as the substrate. The results are expressed as arithmetic mean plus/minus SEM of triplicate dishes. [a] $P < 0.01$ comparison with RAW264.7 cells incubated without AsBe. [b] $P < 0.001$.

The cellular lysosomal enzyme (acid phosphatase) activity in RAW264.7 cells was assayed by a previously described method using *p*-nitrophenyl phosphate as the substrate.[8] As a result, AsBe did not significantly affect the activity in the presence or absence of rMu IFNγ (10 U cm^{-3}) plus LPS (100 ng cm^{-3}) (Fig. 4).

RAW264.7 cells were incubated with 10 mmol dm^{-3} AsBe for 1 to 48 h and the cellular GSH levels were measured by a method described elsewhere using *o*-phthaldialdehyde as the substrate.[9] As shown in Fig. 5, AsBe temporarily increased the cellular GSH levels with a peak at 6 h after the incubation, but levels then decreased from 12 to 24 h. They later recovered again to the control level at 48 h. The same results were obtained through the incubation with 1 mmol dm^{-3} AsBe (data not shown).

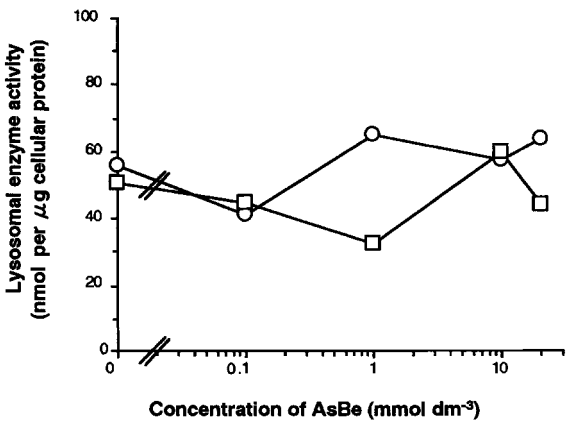

Figure 4. Effect of AsBe on lysosomal enzyme activity in RAW264.7 cells. The RAW264.7 cells were incubated with AsBe in the presence of 10 U cm^{-3} rMu IFNγ plus 100 ng cm^{-3} LPS (□) or medium alone (○) for 48 h at 37 °C, and the cellular lysosomal enzyme (acid phosphatase) activity was measured by a previously described method using *p*-nitrophenyl phosphate as the substrate. The results are expressed as arithmetic mean plus/minus SEM of triplicate dishes.

Effect of AsBe on the viability and the cellular GSH levels of rat liver TRL1215 cells and human skin TIG-112 cells

We examined the cytotoxic effects of AsBe using other cells, such as rat liver TRL1215 cells and human skin TIG-112 cells. It is known that the liver is the major site of arsenic methylation and it is also a target tissue of arsenic toxicity and carcinogenicity,[10] and it was determined that skin cancer is induced by inorganic arsenic compounds in humans.[10] TRL1215 cells and TIG-112 cells were incubated with various concentrations of AsBe for 48 h at 37 °C in a CO_2 incubator in the presence or absence of BSO (1 mmol dm^{-3}), and the viability of the cells was investigated by the AB assay or CV assay. The results showed that AsBe had no cytotoxity on either TRL1215 cells or TIG-112 cells, even at concentrations over 20 mmol dm^{-3}, with or without BSO as determined by the AB assay (Figs 6 and 7). BSO had a low cytoxicity on TIG-112 cells (Fig. 7). The same results were found using the CV assay (data not shown). Subsequently, we measured the cellular GSH levels of these cells. As shown in Figs 8 and 9, AsBe temporarily increased the cellular GSH of both the TRL1215 cells and the TIG-112 cells, with a peak at 6 h after the incubation, and it recovered to the control

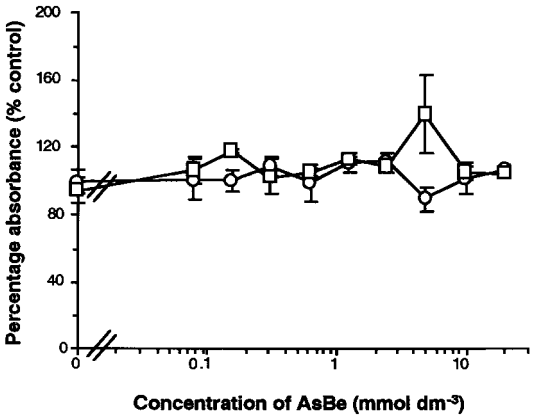

Figure 6. Cytotoxicity of AsBe in rat liver TRL1215 cells. The TRL1215 cells were incubated with AsBe in the presence of 1 mmol dm^{-3} BSO (□) or mediun alone (○) for 48 h at 37 °C, and the cytotoxicity of the cells was determined by the AB assay. One representative experiment out of three similar ones is shown. The results are expressed as arithmetic mean plus/minus SEM of triplicate dishes.

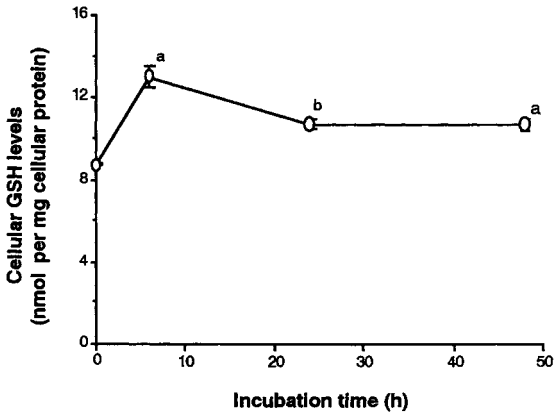

Figure 8. Effect of AsBe on the cellular GSH levels of TRL1215 cells. The TRL1215 cells were incubated for various exposure times with 10 mmol dm^{-3} AsBe at 37 °C, and the cellular GSH levels were measured by a method described elsewhere using *o*-phthaldialdehyde as the substrate. One representative experiment out of three similar ones is shown. The results are expressed as arithmetic mean plus/minus SEM of triplicate dishes. [a] $P < 0.01$ comparison with TRL1215 cells incubated without AsBe. [b] $P < 0.001$.

level at 24 h. The same results were obtained with the incubation of 1 mmol dm^{-3} AsBe (data not shown).

DISCUSSION

Marine animals are ingested daily as seafood in many countries and some contain very high concentrations of arsenic.[1] These arsenicals are generally in the form of water-soluble organic arsenic compounds, and the trimethyl (carboxymethyl) arsonium zwitterion, namely AsBe, was found as a major arsenic compound in marine animals.[3] In the marine ecosystem, it has been demonstrated that inorganic arsenicals in sea water are probably first taken up into seaweed and are metabolically methylated to

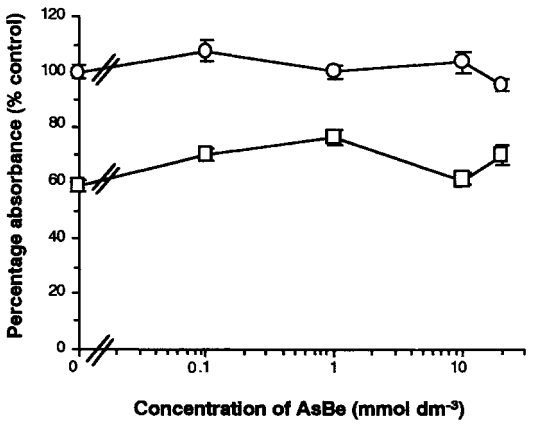

Figure 7. Cytotoxicity of AsBe in human skin TIG-112 cells. The TIG-112 cells were incubated with AsBe in the presence of 1 mmol dm^{-3} BSO (□) or mediun alone (○) for 48 h at 37 °C, and the cytotoxicity of cells was determined by the AB assay. One representative experiment out of three similar ones is shown. The results are expressed as arithmetic mean plus/minus SEM of triplicate dishes.

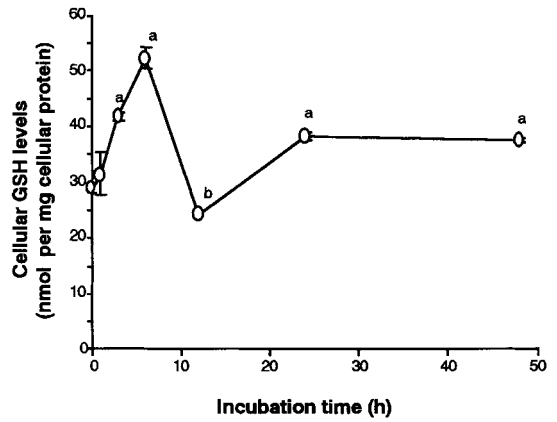

Figure 9. Effect of AsBe on the cellular GSH levels of TIG-112 cells. The TIG-112 cells were incubated for various exposure times with 10 mmol dm^{-3} AsBe at 37 °C, and the cellular GSH levels were measured by a method described elsewhere using *o*-phthaldialdehyde as the substrate. One representative experiment out of two similar ones is shown. The results are expressed as arithmetic mean plus/minus SEM of triplicate dishes. [a] $P < 0.001$ comparison with TIG-112 cells incubated without AsBe. [b] $P < 0.01$.

dimethylarsinoyl ribosides (arsenosugars).[11] The arsenosugars are further methylated and converted to AsBe in many species of marine animals.[1,3] It has been suggested that AsBe is the final metabolite of arsenicals in this ecosystem. Thus, it is very important for us to study the cytotoxicity of AsBe, because we daily ingest AsBe in seafood. In this study, we examined whether AsBe has any cytotoxicity *in vitro* on various kinds of mammalian cells, *viz.* mouse macrophage RAW264.7 cells, rat liver TRL1215 cells and human skin TIG-112 cells.

These cells were incubated with various concentrations of AsBe for 48 h at 37 °C in a CO_2 incubator in the presence or absence of BSO. The results showed that AsBe was not toxic *in vitro* in RAW264.7 cells, TRL1215 cells and TIG-112 cells, even at concentrations over 20 mmol dm^{-3} and whether or not cellular GSH was depleted by the addition of BSO (Figs 1, 6 and 7). AsBe also did not significantly affect various immune functions of the RAW264.7 cells, such as the release of IL-1α and the cellular lysosomal enzyme activity (Figs 3 and 4). Only NO_2^- production was inhibited by a very high concentration of AsBe (20 mmol dm^{-3}) (Fig. 2). We have also demonstrated that the toxicity of AsBe in mammals was very weak. It was reported that AsBe had no acute toxicity when it was administered orally to mice,[4] and we previously described that AsBe was less toxic *in vitro* in murine macrophages, splenocytes, thymocytes and Payer's patch lymphocytes, even at concentrations over 10 mmol dm^{-3}.[12,13] Thus, we perceived that AsBe did not have any cytotoxic effects in mammals. In this study, however, AsBe showed an effect in the cellular GSH levels, which are known to be sensitively changed by toxic compounds, including inorganic arsenicals.[14] AsBe temporarily increased the cellular GSH levels, with a peak at 6 h incubation, and then it recovered to the control level at 48 h (Figs 5, 8 and 9). This suggested that the observations of the changes of the cellular GSH levels by chemical stimulation may be very useful in evaluating any unknown chemical cytotoxicity, because the cellular GSH level was changed even by the stimulation of AsBe, which is the least toxic arsenical. It also indicated that cellular GSH plays an important role in protecting the cells from the weak toxicity of AsBe exposure.

We recently reported that AsBe showed a unique biological effect on murine bone marrow (BM) cells *in vitro*; AsBe significantly enhanced the initial adhesion abilities and viability of immature mouse BM cells and subsequently increased the continuous survival of large spreading cells, especially granulocytes and macrophages, that originate from the BM cells.[13] It indicated that AsBe has a possible application as a biological response modifier (BRM) to increase the number of granulocytes and macrophages. We previously reported that AsBe had no acute toxicity and immunotoxicity,[12,13] and AsBe had no cytotoxicity in mouse macrophage RAW264.7 cells, rat liver TRL1215 cells and human skin TIG-112 cells in this study. Thus, there is a suggestion that it might be possible to use AsBe as a BRM without fatal toxic side effects. The reason why AsBe only shows expression of significant effects on the viability of immature BM cells has not yet been clarified. We postulated that the effect of AsBe in BM cells might be direct rather than due to autocrine mechanisms.[13] We also suggest that the biological effect in BM cells induced by AsBe may depend on the conformational changes in the cellular proteins, which contain thiol groups, because the cellular GSH levels were transiently changed by the AsBe treatment in this study (Figs 5, 8 and 9).

In conclusion, we have demonstrated that AsBe has no cytotoxicity for the various mammal cells examined, *viz.* mouse macrophage RAW264.7 cells, rat liver TRL1215 cells and human skin TIG-112 cells. AsBe is a major organic arsenic compound in marine animals that are ingested daily as seafood in many countries. Thus, the study has significance in food hygiene.

Acknowledgements

This work was partly supported by the Takeda Science Foundation, Japan. We express our thanks to Dr Masumi H. Sakurai for the limulus test and help with the preparation of this manuscript, and Mr Akira Kurihara and Mr Takami Ohta for their excellent technical assistance.

REFERENCES

1. Kaise T, Hanaoka K, Tagawa S, Hirayama T and Fukui S. *Appl. Organomet. Chem.* 1988; **2**: 539.
2. Le X-C, Cullen WR and Reimer KJ. *Clin. Chem.* 1994; **40**: 617.
3. Edmonds JS, Francesconi KA, Cannon JR, Raston CR, Skelton BW and White AH. *Tetrahedron Lett.* 1977; **18**: 1543.
4. Kaise T, Watanabe S and Itoh K. *Chemosphere* 1985; **14**: 1327.
5. Romach EH, Zhao CQ, Razo LMD, Cebrian ME and Waalkes MP. *Toxicol. Sci.* 2000; **54**: 500.
6. Kondo H and Yonezawa Y. *Exp. Cell. Res.* 1995; **220**: 501.
7. Corradin SB and Mauel J. *J. Immunol.* 1991; **146**: 279.
8. Sakurai T, Kaise T, Yadomae T and Matsubara C. *Eur. J. Pharmacol.* 1997; **334**: 255.
9. Hissin PJ and Hilf R. *Anal. Biochem.* 1976; **74**: 214.
10. National Research Council. *Arsenic in the Drinking Water*. National Academy Press: Washington, DC, 1999.
11. Edmonds JS and Francesconi KA. *Nature* 1981; **289**: 602.
12. Sakurai T, Kaise T and Matsubara C. *Appl. Organomet. Chem.* 1996; **10**: 727.
13. Sakurai T and Fujiwara K. *Br. J. Pharmacol.* 2001; **132**: 143.
14. Taketani S, Sato H, Yoshinaga T, Tokunaga R, Ishii T and Bannai S. *J. Biochem.* 1990; **108**: 28.

