

## Effects of Arsenic Compounds on Growth, Cell-Cycle Distribution and Apoptosis of Tretinoin-resistant Human Promyelocytic Leukemia Cells

CHIZUKO SAKAI<sup>1</sup>, MARIKO ARAI<sup>2</sup>, SACHIKO TANAKA<sup>1</sup>, KENJI ONDA<sup>1</sup>,  
KENTARO SUGIYAMA<sup>1</sup> and TOSHIHIKO HIRANO<sup>1</sup>

<sup>1</sup>Department of Clinical Pharmacology, School of Pharmacy,  
Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo, Japan;

<sup>2</sup>Department of Pharmacy, Kitasato University East Hospital, Sagamihara, Kanagawa, Japan

**Abstract.** *Background/Aim:* The effects of inorganic and organic arsenicals on proliferation, cell-cycle distribution, and apoptosis of all-transretinoic acid (ATRA)-resistant human promyelocytic leukemia HL-60 (HL-60-R2) cells were herein investigated. *Materials and Methods:* Cell proliferation was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Cell-cycle distribution and apoptotic cells were analyzed by flow cytometry. *Results:* The 50% inhibitory concentrations ( $IC_{50}$  values) for  $As_2O_3$  against proliferation of HL-60 and HL-60-R2 cells were 12.2 and 7.2  $\mu M$ , while those for arsenate were >200 and 62.1  $\mu M$ , respectively. In contrast, organic methylarsinic acid, dimethylarsonic acid, trimethylarsine oxide, and tetramethylarsonium did not exert any inhibitory effects even at 200  $\mu M$ .  $As_2O_3$  and arsenate increased the proportion of apoptotic cells dose-dependently at a concentration range of 5-200  $\mu M$ .  $As_2O_3$  did not activate caspase 3/7 in HL-60 and HL-60-R2 cells. *Conclusion:*  $As_2O_3$  and arsenate inhibit cell proliferation, affect cell-cycle distribution, and induce apoptosis of ATRA-resistant HL-60-R2 cells. The apoptosis-inducing mechanism appears not to be mediated through caspase 3/7.

Arsenicals are natural substances that have been used medicinally for over 2,400 years. Recent clinical studies have shown that  $As_2O_3$  is an effective and relatively safe drug in the treatment of acute promyelocytic leukemia (APL) (1-3).  $As_2O_3$  is also effective for patients with APL who are

resistant to all-transretinoic acid (ATRA) and conventional chemotherapy (4, 5). It has been reported that the effects of  $As_2O_3$  are not limited to APL cells but can be observed in other cells of either myeloid (6), lymphoid (7) or drug-resistant sublines (8). Anti-proliferative and anti-invasive effects of inorganic and organic arsenic compounds were suggested in human and murine melanoma cells *in vitro* (9).

The action mechanisms of  $As_2O_3$  in APL and other malignancies are thought to be due to inhibition of growth and induction of apoptosis (6-8, 10). Glutathione depletion enhances  $As_2O_3$ -induced apoptosis of cancer cells, suggesting that the antineoplastic efficacy of  $As_2O_3$  relates to an increase in oxidative stress (11-14). Furthermore, selective sensitivity of arsenic compounds to dividing endothelial cells, which results in inhibition of angiogenesis, raise the possibility for designing better targeted- antineoplastic arsenic-containing compounds with less toxicity to normal cells (15). Although mechanisms of the anticancer actions of  $As_2O_3$  have been studied as described above, the inhibitory effects of  $As_2O_3$  and many other organic and inorganic arsenic-compounds on ATRA-resistant APL cells have been scarcely studied.

Thus, the present study was undertaken to evaluate anti-proliferative effects of inorganic and organic arsenic compounds on APL-related leukemia cell line HL-60 resistant to ATRA (HL-60-R2) (16), as well as the parental HL-60 cells, and to provide insight into their mechanisms of action by examining cell growth, apoptosis, and caspase 3/7 activity in these cells.

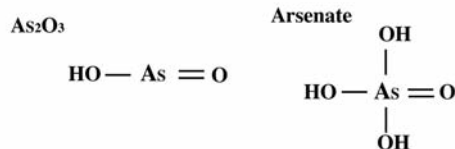
### Materials and Methods

**Materials.** RPMI-1640 medium and fetal bovine serum were purchased from Gibco BRL Co. (Grand Island, NY, USA).  $As_2O_3$ , sodium arsenate, sodium methylarsonate, dimethylarsinic acid, trimethylarsine oxide, and tetramethylarsonium were purchased from Tri Chemical Laboratories Inc. (Yamanashi, Japan). The chemical structures of these arsenic compounds are shown in Figure 1. Stock solutions of these agents were made at a concentration of 10 mmol/l and diluted to working concentrations by distilled water before use. Cell proliferation

*Correspondence to:* Toshihiko Hirano, Ph.D., Department of Clinical Pharmacology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan. Tel: +81 426765794, Fax: +81 426765798, e-mail: hiranot@toyaku.ac.jp

**Key Words:** Arsenic trioxide, arsenate, organic arsenicals, HL-60 cells, all-trans-retinoic and resistance, apoptosis induction.

# Inorganic arsenic compounds



# Organic arsenic compounds

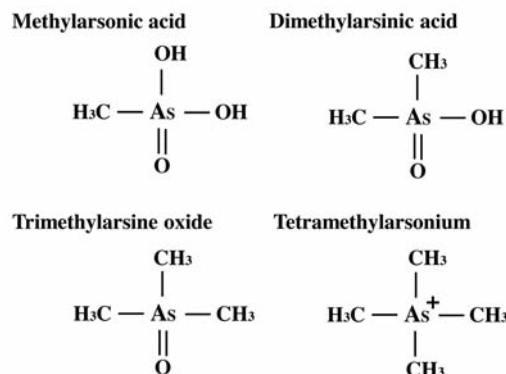


Figure 1. Chemical structures of inorganic and organic arsenic compounds used in the study.

Kit I and II (MTT) were purchased from Roche Diagnostics Co. (Indianapolis, IN, USA). Annexin V-FITC Apoptosis Detection KitI were products of BD PharMingen (San Diego, CA, USA).

**Cell culture and cell proliferation assay.** HL-60-R2 cells were established by exposing parental HL-60 cells to stepwise increasing concentrations (from 100 pM) of ATRA for two months (16). These cell lines were obtained from Riken BioResource Center, Tsukuba, Japan. The leukemia cells were washed and resuspended with the above medium to  $5 \times 10^5$  cells/ml, then 98  $\mu\text{l}$  of the cell suspension were placed in each well of a 96-well flat-bottom plate. Two microliters of ethanolic solution containing each test compound or 2  $\mu\text{l}$  of ethanolic solution containing ATRA were added to yield final concentrations of 0.001, 0.01, 0.1, 1, 10 and 100  $\mu\text{mol/l}$ , respectively. Two microliters of ethanol were added to the control wells. The cells were incubated for 72 h in a humidified chamber with 5%  $\text{CO}_2$ /air at 37°C.

After the incubation period, 15  $\mu\text{l}$  of the MTT labeling reagent (final concentration of 0.5 mg/ml) were added into each well and the plate was mixed on a microshaker for 10 s. The cells were then incubated for a further 4 h in a humidified atmosphere. Subsequently, 100  $\mu\text{l}$  of solubilization solution were added to each well, and the plate was mixed on a microshaker for 10 s and allowed to stand overnight in the incubator in a humidified atmosphere. The spectrophotometrical absorbance of the sample was measured on a microplate reader (Corona MT P-32; Corona Co., Hitachi, Ibaraki, Japan) at 570 nm. A dose-response curve was plotted for each drug, and a concentration which yielded a 50% inhibition of cell growth ( $\text{IC}_{50}$ , ng/ml) was calculated. The  $\text{IC}_{50}$  values were determined from linear regression of at least four points at different concentrations of the agents.

**Cell-cycle analysis.** HL-60 and HL-60-R2 cells were washed and resuspended with the above medium to  $5 \times 10^5$  cells/ml, and then

980  $\mu\text{l}$  of the cell suspension were placed in each well of a 24-well flat-bottom plate. Subsequently, 20  $\mu\text{l}$  of  $\text{As}_2\text{O}_3$  solution were added to give final agent concentrations of 0.2-200  $\mu\text{M}$ . After culturing of the cells for 72 h in 5%  $\text{CO}_2$ /air at 37°C, cells were placed in 1.5 ml tubes, washed twice in phosphate-buffered saline containing 1% fetal bovine serum, and then fixed in 70% ethanol at -20°C for 24 h. After fixation, the cells were washed twice with the above buffer, and treated with 1 mg/ml of RNase at 37°C for 30 min. Subsequently, 10  $\mu\text{l}$  of propidium iodide solution (Japan Becton Dickinson, Minatoku, Tokyo, Japan) was added to this cell suspension ( $5 \times 10^5$  cells/60  $\mu\text{l}$ ), and the tube containing the cell suspension was incubated for 20 min at room temperature in the dark. Subsequently, the cells were resuspended with 800  $\mu\text{l}$  of phosphate-buffered saline containing 0.4% paraformaldehyde and 1% fetal bovine serum, and were analyzed by flow cytometry (Becton Dickinson) within one hour after staining. A total of 30,000 non-gated cells were analyzed.

**Caspase-3/7 activity assay.** Leukemia cells were incubated with the test agents for 4, 12, and 24 h, respectively. Thereafter, 100  $\mu\text{l}$  of Caspase-Glo 3/7 (Promega, Madison, WI, USA) reagent were added to each sample, and the cells were incubated for 30 min and assayed (8) according to the manufacturer's instructions. The data were analyzed with GraphPad Prism 4.0 (GraphPad Software Inc., La Jolla, CA 92037 USA).

**Statistics.** Comparison of the data between the two groups was carried-out by unpaired Student's *t*-test. In each case, *p*-values less than 0.05 were considered significant.

## Results

**Effects of inorganic and organic arsenic compounds on cell proliferation of human leukemia cells.** Effects of two inorganic and four organic arsenic compounds on cell proliferation and apoptosis of human promyelocytic leukemia HL-60 cells and ATRA-resistant HL-60 (HL-60-R2) cells were examined *in vitro*. These cells were incubated in the presence of serial concentrations of the agents for 72 h, and then were analyzed with MTT assay procedures.

Decreased sensitivity of HL-60-R2 cells to the growth-inhibitory effects of ATRA, compared to its parental HL-60 cells, was demonstrated, as shown in Figure 2. Statistically significant differences of the effects of ATRA between these two cell lines were observed at concentrations of 0.1, 1, and 10  $\mu\text{M}$  ( $p < 0.05$ ). Inorganic arsenicals,  $\text{As}_2\text{O}_3$  and arsenate, dose-dependently inhibited the proliferation of HL-60 cells and HL-60-R2 cells (Figure 3). HL-60-R2 cells were rather sensitive to the growth-inhibitory effects of these inorganic compounds compared to the parental HL-60 cells. Statistically significant differences in the effects of  $\text{As}_2\text{O}_3$  and arsenate between these two cell lines were observed at concentrations of 10  $\mu\text{M}$  for  $\text{As}_2\text{O}_3$  ( $p = 0.004$ ) (Figure 3A) and 200  $\mu\text{M}$  for arsenate ( $p < 0.001$ ) (Figure 3B), respectively. The  $\text{IC}_{50}$  values for  $\text{As}_2\text{O}_3$  against the growth of HL-60 cells and HL-60-R2 cells were 12.2, and 7.2  $\mu\text{M}$ , respectively (Table I), while those for arsenate were >200 and 62.1  $\mu\text{M}$ , respectively (Table I). In

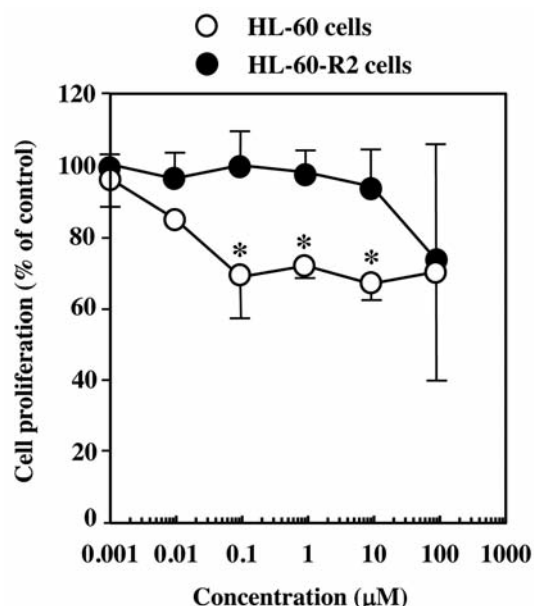


Figure 2. Differential sensitivity of HL-60 and HL-60-R2 cells to the growth-inhibitory effect of all-trans-retinoic acid. Cells were cultured in the presence of serial concentrations of all-trans-retinoic acid for 72 h, and the proliferating cells were analyzed by the MTT assay. Bars indicate the mean $\pm$ SD of three independent experiments. Significant difference at  $*p<0.05$  between the two cell lines.

contrast, the organic compounds methylarsonic acid, dimethylarsinic acid, and trimethylarsine oxide, and tetramethylarsonium did not have any apparent inhibitory effect on HL-60 cells even at 200  $\mu$ M (Figure 4). We also examined the effects of methylarsonate on the proliferation of HL-60-R2 cells, but this organic compound did not have any suppressive effects, having an  $IC_{50}$  value of more than 20  $\mu$ M (Table I).

**Apoptosis induction and caspase 3/7 activities in leukemia cells treated with inorganic and organic arsenic compounds.** As described above,  $As_2O_3$  and arsenate inhibited proliferation of the parental and ATRAn-resistant HL-60 cells, while the four organic arsenicals did not have any significant effect on the cell proliferation. We further examined the effects of inorganic arsenicals on apoptosis and caspase 3/7 activities in these cell lines. HL-60 cells and HL-60-R2 cells were treated with serial concentrations of  $As_2O_3$  for 72 h and stained with propidium iodide. Subsequently, cell-cycle analysis was performed with flow cytometry (Figure 5).  $As_2O_3$  at concentrations of 2-200  $\mu$ M dose-dependently increased the percentage of apoptotic sub- $G_1$  cells, whereas this agent reduced the percentage of G1 phase cells in both HL-60 and HL-60-R2 cells (Figure 5).

Caspase 3/7 activities in HL-60 and HL-60-R2 cells treated with 10 or 20  $\mu$ M  $As_2O_3$  for 4, 12, and 24 h, respectively, are

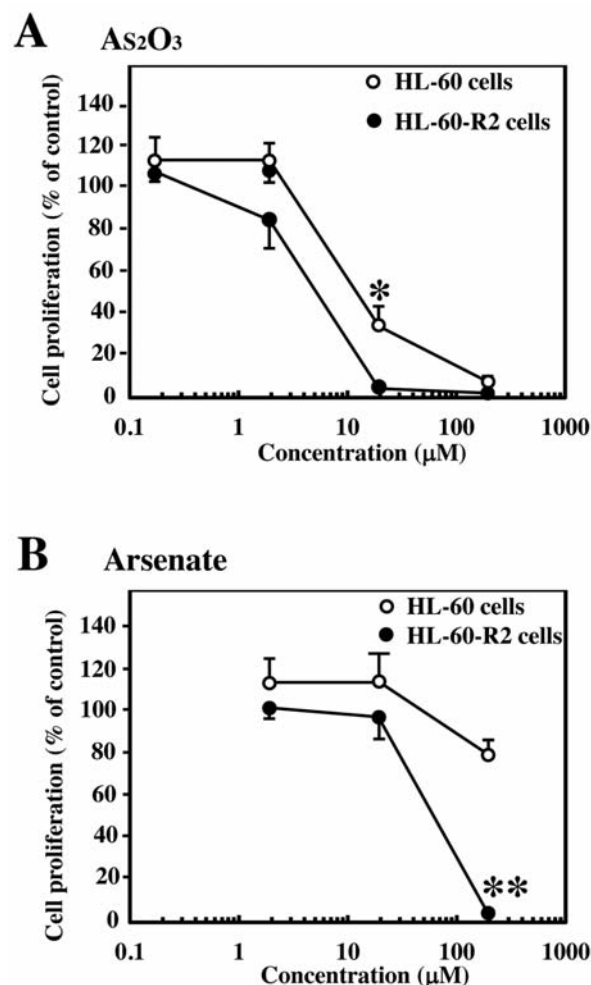


Figure 3. Dose-response curves for  $As_2O_3$  (A) and arsenate (B) against the proliferation of HL-60 and HL-60-R2 cells. Cells were cultured in the presence of serial concentrations of the agents for 72 h, and the proliferating cells were analyzed by the MTT assay. Significant different at  $*p=0.004$  and  $**p<0.001$  between the two cell lines.

shown in Figure 6. While at these concentrations  $As_2O_3$  increased the percentage of apoptotic cells as shown in Figure 5, it did not change caspase 3/7 activities in these cells for any incubation time examined (Figure 6).

## Discussion

The data described above show that inorganic arsenicals  $As_2O_3$  and arsenate inhibit proliferation of both HL-60 and the ATRA-resistant HL-60-R2 cells, whereas the four organic arsenicals methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide, and tetramethylarsonium are not effective against the proliferation of HL-60 cells nor HL-60-R2 cells. Although the precise mechanism of action of these compounds remains to be elucidated,  $As_2O_3$  and arsenate are

Table I.  $IC_{50}$  values ( $\mu M$ ) for arsenic compounds on proliferation of HL-60 and HL-60-R2 cells.

Compound	HL-60	HL-60-R2
Inorganic		
As <sub>2</sub> O <sub>3</sub>	12.21	7.22
Arsenate	>200	62.14
Organic		
Methylarsonate	>20	>20
Dimethylarsinic acid	>20	NE
Trimethylarsine oxide	>20	NE
Tetramethylarsonium	>20	NE

NE: Not examined.

suggested to affect the cell cycle and induce apoptosis in these cell lines without changing caspase 3/7 activities.

We have reported that As<sub>2</sub>O<sub>3</sub> and arsenate have anti-proliferative effects on both T-lymphoblastoid leukemia MOLT-4 cells and daunorubicin-resistant MOLT-4/DNR cells expressing functional P-glycoprotein (8). These observations suggest that As<sub>2</sub>O<sub>3</sub> and arsenate might not be substrates of P-glycoprotein, and thus these agents are effective against multidrug-resistant T-lymphoblastoid leukemia cells expressing functional P-glycoprotein (8). In contrast, the organic arsenicals methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide, and tetramethylarsonium were not effective against the proliferation of MOLT-4 cells and daunorubicin-resistant MOLT-4/DNR cells (8). Suppressive efficacies of As<sub>2</sub>O<sub>3</sub> and arsenate on *in vitro* proliferation of HL-60 cells were also described by Charoensuk *et al.* (17), but the effects of these inorganic arsenicals on ATRA-resistant HL-60 cells have not been examined. The HL-60-R2 cell line was established from human promyelocytic leukemia HL-60 cells by exposing the parental HL-60 cells to increasing concentrations of ATRA (16). ATRA is known to inhibit proliferation, promote apoptosis, and induce differentiation of HL-60 cells, whereas HL-60-R2 cells are resistant to all effects of ATRA (16). The molecular mechanism underlying the ATRA resistance in HL-60-R2 cells, however, is not well understood. It is, therefore, noteworthy that the anti-proliferative effects of As<sub>2</sub>O<sub>3</sub> at 20  $\mu M$  and arsenate at 200  $\mu M$  are significantly greater on the ATRA-resistant HL-60-R2 cells than on the parental HL-60 cells in the present study (Figure 3). When we compare the  $IC_{50}$  values of these inorganic arsenicals, the effects on the resistant HL-60-R2 cells were 1.69- to 3.22-times greater than those on the parental HL-60 cells (Table I). These results suggest that As<sub>2</sub>O<sub>3</sub> is useful for the patients with APL showing resistance to ATRA therapy, which is actually relevant to the clinical efficacy of As<sub>2</sub>O<sub>3</sub>. Studies on clinical pharmacokinetics of As<sub>2</sub>O<sub>3</sub> have shown that the peak level of plasma As<sub>2</sub>O<sub>3</sub> concentration under a general treatment schedule was 5.5 to 7.3  $\mu M$  (18). The  $IC_{50}$  values for As<sub>2</sub>O<sub>3</sub> against the proliferation of HL-60 cells and HL-60-R2

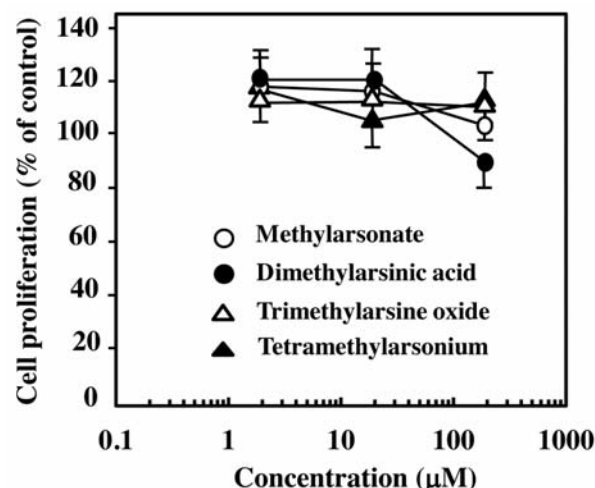


Figure 4. Effects of four organic arsenic compounds (see Figure 1) on the proliferation of HL-60 cells. The cells were cultured in the presence of serial concentrations of the agents for 72 h, and the proliferating cells were analyzed with MTT assays. Bars indicate the mean  $\pm$  SD of three independent experiments.

cells were 12.21 and 7.22  $\mu M$ , respectively (Table I), which are near peak plasma levels after administration of the agent to patients with leukemia.

Our present study suggests that As<sub>2</sub>O<sub>3</sub> affects the cell cycle and induces apoptosis of both HL-60 and HL-60-R2 cells, probably *via* a cellular signaling pathway independent of caspase 3/7. We have reported that As<sub>2</sub>O<sub>3</sub> influences the cell cycle and induces apoptosis of MOLT-4 and MOLT-4/DNR cells *via* activation of caspase-3 (11). These discrepant effects of As<sub>2</sub>O<sub>3</sub> on caspase-3 induction might be due to the difference in leukemia cell lines. Charoensuk *et al.* reported that an increase in caspase-3 activity in HL-60 cells was observed by treatment of cells with certain organic arsenicals (17), other than those we examined in this study. These data imply that certain kinds of organic arsenicals induce apoptosis of HL-60 cells through a caspase-dependent mechanism. They also showed that As<sub>2</sub>O<sub>3</sub> and arsenate failed to activate caspase-3, suggesting that these inorganic arsenicals induce apoptosis by an alternative pathway (17). Our present data are consistent with their observations, and thus further studies are required for disclosing the molecular mechanism of the apoptosis-inducing effect of these inorganic arsenicals in ATRA-resistant HL-60-R2 cells. As<sub>2</sub>O<sub>3</sub> has been reported to induce oxidative stress, DNA damage, and the mitochondrial pathway of apoptosis in several human cancer cell lines (19-21). These observations suggest that As<sub>2</sub>O<sub>3</sub> induces apoptosis of ATRA-resistant HL-60-R2 cells through similar mechanisms related to oxidative stress induction.

The present study, in summary, showed that inorganic arsenic compounds, As<sub>2</sub>O<sub>3</sub> and arsenate, inhibit cell proliferation, affect the cell cycle, and induce apoptosis of



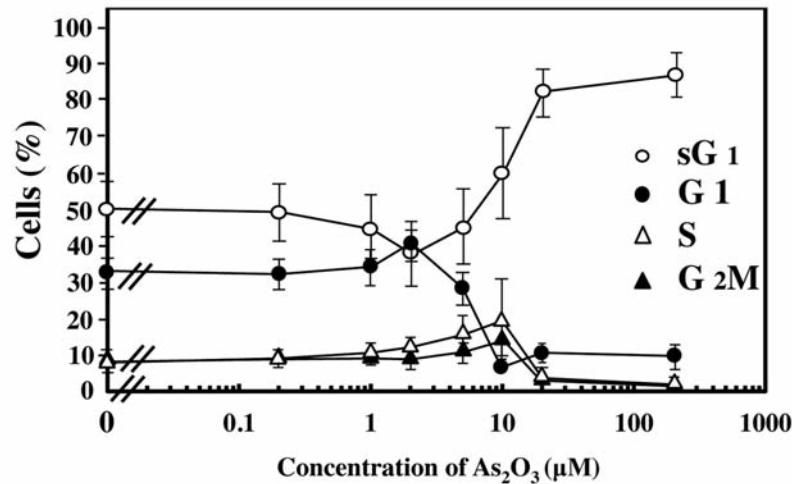
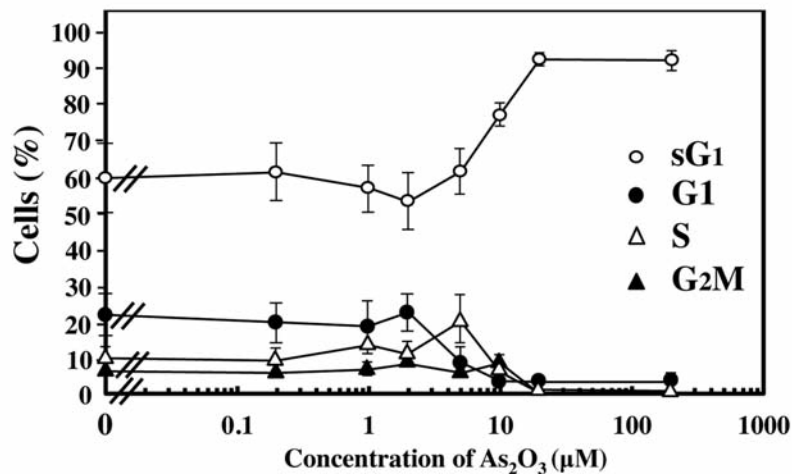
**A HL-60 cells****B HL-60-R2 cells**

Figure 5. Cell-cycle distribution of HL-60 (A) and HL-60-R2 (B) cells after  $As_2O_3$  treatment. Cells were treated with 0.2-200  $\mu M$  of  $As_2O_3$  for 72 h, stained with propidium iodide, and analyzed by flow cytometry. The percentages of sub- $G_1$  (sG1; apoptotic),  $G_1$ , S, and  $G_2M$  phase cells in the cell cycle are shown. Each bar represents the mean $\pm$ SD of three independent experiments.

promyelocytic leukemia HL-60 cells and the ATRA-resistant HL-60-R2 cells. In contrast, organic compounds methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide, and tetramethylarsonium did not have any inhibitory effects on these cells. The apoptosis-inducing mechanism of  $As_2O_3$  appears not to be mediated through caspase3/7, although the precise action mechanism of  $As_2O_3$  remains to be elucidated.

**Conflicts of Interest**

The Authors declare that there are no conflicts of interest in this study.

**References**

- 1 Li J, Zhu H, Hu J, Mi J, Chen S, Chen Z and Wang Z: Progress in the treatment of acute promyelocytic leukemia: optimization and obstruction. *Int J Hematol* 100: 38-50, 2014..
- 2 Nichol JN, Garnier N and Miller WH Jr.: Triple A therapy: The molecular underpinnings of the unique sensitivity of leukemic promyelocytes to anthracyclines, all-*trans*-retinoic acid and arsenic trioxide. *Best Pract Res Clin Haematol* 27: 19-31, 2014.
- 3 Sanz MA, Iacoboni G and Montesinos P: Conventional induction and post-remission therapy in APL: Have we arrived? *Best Pract Res Clin Haematol* 27: 33-38, 2014.

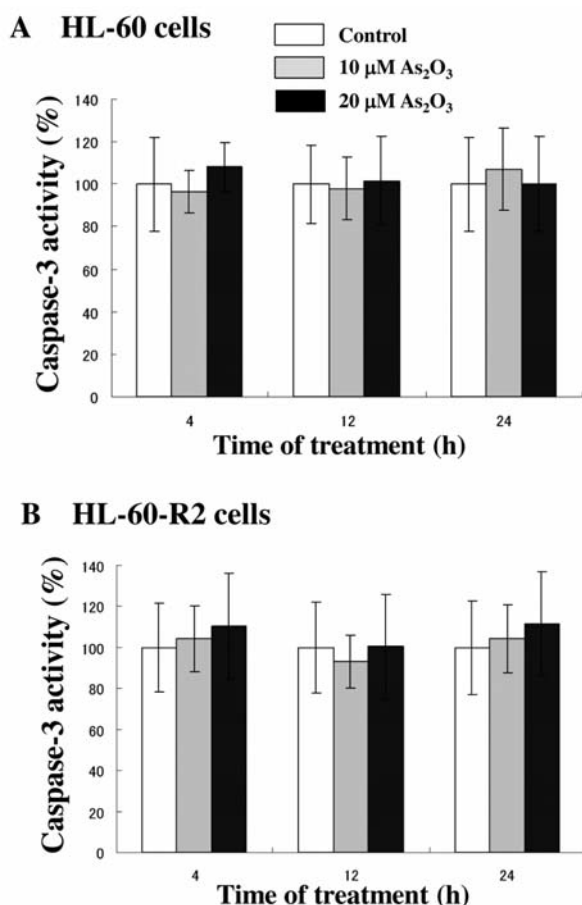


Figure 6. Caspase 3/7 activities in HL-60 (A) and HL-60-R2 (B) cells treated by  $As_2O_3$ . Cells were cultured in the presence of 10 or 20  $\mu$ M  $As_2O_3$  for 4, 12, and 24 h, respectively, and cellular caspase 3/7 activities were analyzed as described in the Materials and Methods. The caspase activities were expressed as a percentage to that of the control. Bars indicate the mean  $\pm$  SD of three independent experiments.

- 4 Douer D: Advances in the treatment of relapsed acute promyelocytic leukemia. *Acta Haematol* 107: 1-17, 2002.
- 5 Lin CP, Huang MJ, Chang IY and Lin WY: Successful treatment of all-*trans* retinoic acid resistant and chemotherapy naïve acute promyelocytic patients with arsenic trioxide – two case reports. *Leuk Lymphoma* 38: 191-194, 2000.
- 6 Xu W, Wei W, Yu Q, Wu C, Ye C, Wu Y and Yan H: Arsenic trioxide and bortezomib interact synergistically to induce apoptosis in chronic myelogenous leukemia cells resistant to imatinib mesylate through BCR/ABL dependent mechanisms. *Mol Med Rep* 10: 1519-24, 2014.
- 7 Zhang XH, Feng R, Lv M, Jiang Q, Zhu HH, Qing YZ, Bao JL, Huang XJ and Zheng XL: Arsenic trioxide induces apoptosis in B-cell chronic lymphocytic leukemic cellsthrough down-regulation of survivin *via* the p53-dependent signaling pathway. *Leuk Res* 37: 1719-1725, 2013.
- 8 Hikita E, Arai M, Tanaka S, Onda K, Utsumi H, Yuan B, Toyoda H and Hirano T: Effects of inorganic and organic arsenicals on growth and apoptosis of human T-lymphoblastoid leukemia cells. *Anticancer Res* 31: 4169-4178, 2011.
- 9 Hiwatashi Y, Tadokoro H, Henmi K, Arai M, Kaise T, Tanaka S and Hirano T: Antiproliferative and anti-invasive effects of inorganic and organic arsenic compounds on human and murine melanoma cells *in vitro*. *J Pharm Pharmacol* 63: 1202-1210, 2011.
- 10 Hu XM, Hirano T and Oka K: Arsenic trioxide induces apoptosis equally to T-lymphoblastoid leukemia MOLT-4 cells and P-gp expressing daunorubicin-resistant MOLT-4 cells. *Cancer Chemother Pharmacol* 51: 119-126, 2003.
- 11 Hu X-M, Hirano T and Oka K: Arsenic trioxide induces apoptosis in MOLT-4 and its daunorubicin-resistant MOLT-4 cell lines *via* depletion of intracellular glutathione, disruption of mitochondrial membrane potential and activation of caspase-3. *Cancer Chemother Pharmacol* 52: 47-58, 2003.
- 12 Han YH, Moon HJ, You BR, Kim SZ, Kim SH and Park WH: Effects of arsenic trioxide on cell death, reactive oxygen species and glutathione levels in different cell types. *Int J Mol Med* 25: 121-128, 2010.
- 13 You BR, Park WH: Arsenic trioxide induces human pulmonary fibroblast cell death *via* increasing ROS levels and GSH depletion. *Oncol Rep* 28: 749-757, 2012.
- 14 Matulis SM, Morales AA, Yehiayan L, Lee KP, Cai Y and Boise LH: Alterations in glutathione levels and apoptotic regulators are associated with acquisition of arsenic trioxide resistance in multiple myeloma. *PLoS One* 7: e52662, 2012.
- 15 Ralph SJ: Arsenic-based antineoplastic drugs and their mechanisms of action. *Met Based Drugs* 2008: 260146, 2008.
- 16 Mori J, Suzuki S, Hara M, Kaneko A, Yamashita K, Kumagai M, Sakuma T, Kakizawa T, Yamazaki M, Takeda T, Miyamoto T, Ichikawa K and Hashizume K: Characterization of two novel retinoic acid-resistant cell lines derived from HL-60 cells following long-term culture with all-*trans*-retinoic acid. *Jpn J Cancer Res* 90: 660-668, 1999.
- 17 Charoensuk V, Gati WP, Weinfeld M and Le XC: Differential cytotoxic effects of arsenic compounds in human acute promyelocytic leukemia cells. *Toxicol Appl Pharmacol* 239: 64-70, 2009.
- 18 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 and Wang ZY: 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, 1997.
- 19 Kumar S, Yedjou CG and Tchounwou PB: Arsenic trioxide induces oxidative stress, DNA damage, and mitochondrial pathway of apoptosis in human leukemia (HL-60) cells. *J Exp Clin Cancer Res* 33: 42, 2014.
- 20 Yen YP, Tsai KS, Chen YW, Huang CF, Yang RS and Liu SH: Arsenic induces apoptosis in myoblasts through a reactive oxygen species-induced endoplasmic reticulum stress and mitochondrial dysfunction pathway. *Arch Toxicol* 86: 923-33, 2012.
- 21 Shen P, Jiang T, Lu H, Han H and Luo R: Combination of Poly I:C and arsenic trioxide triggers apoptosis synergistically *via* activation of TLR3 and mitochondrial pathways in hepatocellular carcinoma cells. *Cell Biol Int* 35: 803-810, 2011.

Received July 9, 2014

Revised August 13, 2014

Accepted August 19, 2014