Cancer Chemother Pharmacol (2001) 47: 481–490
DOI 10.1007/s002800100278

ORIGINAL ARTICLE

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Arsenic compounds induce cytotoxicity and apoptosis in cisplatin-sensitive and -resistant gynecological cancer cell lines

Received: 8 September 2000 / Accepted: 25 January 2001 / Published online: 2 March 2001

Abstract *Purpose*: Arsenic compounds have been found to be effective in the treatment of acute promyelocytic leukemia through the downregulation of bcl-2 expression. Resistant ovarian cancer cells often overexpress bcl-2 or p53 proteins or both. We hypothesized that arsenic compounds, such as As_2O_3 and As_2S_3, could also be active against gynecological cancers resistant to conventional chemotherapy. *Methods*: We investigated the effects of these two arsenic compounds in vitro on ovarian cancer cell lines sensitive (OVCAR, GG, JAM) and resistant (CI80-13S) to cisplatin (CDDP) and on human cervical cancer cell lines (HeLa) in comparison with their effects on human fibroblasts (HF). A fluorometric assay based on measurements of fluorescein diacetate (FDA) in cells was used to determine cell viability. Apoptosis was assessed in terms of cell morphology, by flow cytometry and by a DNA fragmentation assay. *Results*: Treatment of each cell line with the As_2O_3 or As_2S_3 led to a marked dose-dependent decrease in cell growth. The IC_{50} of the two compounds indicated a significantly greater cytotoxic effect against all the cancer cells tested than against the normal HF. At a clinically achievable concentration (2 μM), As_2O_3 selectively inhibited the growth and induced apoptosis in CI80-13S, OVCAR and HeLa cells but had no significant apoptotic effect on GG or JAM cells or HF. Following treatment with 5 μM As_2S_3, the CI80-13S, OVCAR and HeLa cells also exhibited growth inhibition and induction of apoptosis. *Conclusions*: Arsenic compounds (As_2O_3 and As_2S_3) can inhibit growth and induce apoptosis in human ovarian and cervical cancer cells at clinically achievable concentrations, indicating that As_2O_3 and As_2S_3 could be effective in the treatment of gynecological cancer.

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Keywords Apoptosis · Arsenic trioxide (As_2O_3) · Arsenic trisulfide (As_2S_3)

Introduction

Arsenic compounds have been identified as comutagens and cocarcinogens in epidemiological studies [4], but historically they have been used to treat patients with acute leukemia and chronic myelogenous leukemia [14]. Recently, arsenic compounds such as arsenic trioxide (As_2O_3) and arsenic disulfide (As_2S_2) that have been utilized in traditional Chinese medicines as anthelmintics or remedies for skin problems, have been demonstrated to have antileukemic effects in vitro [5–7, 46]. These compounds have been found to be remarkably effective clinically in the treatment of patients with acute promyelocytic leukemia (APL) [19, 34, 36, 37, 42]. As_2O_3 has been shown to be particularly active against refractory APL resistant to both cytotoxic chemotherapy and differentiation therapy by all-*trans*-retinoic acid [6]. Importantly, no significant bone marrow suppression has been noted in most patients following administration of As_2O_3 [19, 34].

The therapeutic efficacy of As_2O_3 in APL has prompted many investigations seeking to elucidate the mechanism of action of As_2O_3 in APL cell lines. Preliminary results have shown that As_2O_3 induces apoptosis in the APL cell line NB4 through downregulation of bcl-2, thus causing degradation of PML (promyelocytic leukemia) and PML-RARα (retinoic acid receptor-α) proteins and expression and activation of the apoptosis-associated protein caspase in the absence of apparent differentiation [1, 2, 5]. More recently, it has been suggested that the antileukemic effects of As_2O_3 are directly related to its ability to induce the relocalization and degradation of PML, as well as the degradation of PML-RARα in APL cells [25, 33, 46].

It has been shown in other studies that the apoptotic effect of As_2O_3 is not specific for APL cells, but can also occur in various other cancer cell lines. At clinically

achievable concentrations of As_2O_3 (1–2 μM) apoptosis is triggered in cells derived from chronic myelogenous leukemia [26], non-M3 acute myelogenous leukemia [24], lymphoid leukemia [44], B-cell leukemia [47], some multiple myeloma [20, 30], human gastric cancer [43], human cervical cancer [45] and some solid tumors, such as esophageal cancer [35] and neuroblastoma [2].

To date, little is known about the effects of arsenic compounds on human ovarian cancer. Ovarian cancer is one of the leading causes of cancer death among women. At present, chemotherapy is the most common treatment. Cisplatin, a DNA-damaging agent, which readily induces apoptosis in vitro, is one of the most widely used anticancer drugs in the treatment of ovarian cancer. Cisplatin-based chemotherapy is highly effective. However, resistance to this drug often occurs and is a major limitation to its clinical use. Experiments using tumor cell lines sensitive and resistant to cisplatin have shown that acquired resistance to the drug is due to one or more factors acting alone or in conjunction with others. These factors include decreased intracellular drug accumulation, increased drug efflux and cellular glutathione levels, loss of DNA mismatch repair, and the activation of certain oncogenes [15, 21, 28].

Drug resistance in tumors can also be caused by the suppression of apoptosis after cytotoxic insult [10]. The expression and activity of p53 and the Bcl-2 family often play important roles in controlling apoptotic responses to drug-induced cellular insults, thus modulating the chemosensitivity of tumor cells [16]. An in vitro study has demonstrated that ovarian carcinoma cell lines resistant to cisplatin naturally overexpress bcl-2 and/or p53 proteins, suggesting a possible important role of these genes in the acquired resistance [12]. As one of the therapeutic effects of As_2O_3 in APL cells is the down-regulation of bcl-2 expression [5], we hypothesized that arsenic compounds might also have effects on ovarian carcinoma.

In this study, we investigated the cytotoxic and apoptotic effects of As_2O_3 and As_2S_3 on four human ovarian cancer cell lines and one cervical cancer cell line, using normal human fibroblasts (HF) as a control. These cell lines have different degrees of sensitivity/resistance to cisplatin. The cytotoxic effects of arsenic compounds on the different cells were determined using a semiautomated fluorometric microculture cytotoxicity assay (FMCA). The apoptotic effects of these arsenic compounds on the cells were further confirmed using phase-contrast microscopy, flow cytometry and a DNA fragmentation assay.

Materials and methods

Cells and cell culture

The cell lines [11] used in our experiments were four human ovarian cancer cell lines (CI80-13S, OVCAR, GG and JAM) and one cervical cancer cell line (HeLa). These cell lines were kindly supplied by Queensland Institute of Medical Research, Australia. The dermal HF used as a normal control were obtained from the Skin Culture Laboratory, Singapore General Hospital. The ovarian cancer cell lines were grown as monolayers in RPMI-1640, and HeLa cells and HF in Dulbecco's modified Eagle's medium (DMEM), supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (HyClone Laboratories, Logan, Utah) at 37°C in a humidified atmosphere containing 5% CO_2. Cell viability of the stock cultures used for subsequent experiments was always above 95% as assessed by trypan blue exclusion.

Drugs and reagents

Stock solutions were prepared by dissolving As_2O_3 and As_2S_3 (Sigma Chemical Company, St Louis, Mo.) in phosphate-buffered saline (PBS) at a concentration of 1.0 mmol/l and stored at 4°C. They were diluted to the working concentrations with culture medium before use. A stock solution of fluorescein diacetate (10 mg/ml, FDA; Sigma) was prepared in DMSO, kept frozen (–20°C) and protected from light.

Cell morphology

Cell morphological changes with or without As_2O_3 or As_2S_3 treatment were assessed by phase-contrast microscopy. Cells were treated with or without the test compounds for 3 days. Photographs of the adherent cells were then taken under a phase-contrast microscope after the medium containing floating cells had been removed.

Fluorometric microculture cytotoxicity assay

The drug cytotoxicity and cell proliferation were determined using a previously described FMCA [22, 23] with minor modification. FMCA is based on measurements of the fluorescence generated from the hydrolysis of FDA to fluorescein by cells with intact plasma membranes. Briefly, cells were seeded into 96-well microtiter plates (10,000 cells per well). The plates were incubated at 37°C overnight to allow the cells to adhere to the bottom. The medium of the plates was then changed to 200 µl medium containing the test compounds or the vehicle of the test compounds as control. After an incubation period of 3 days, the plates were washed with PBS. The FDA stock solution was diluted to 2 µg/ml with PBS and 200 µl of this solution was then added to each of the control, experimental and blank wells. The plates were incubated for 30 min at 37°C and the fluorescence generated from each well was measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. In a preliminary study the fluorescence was found to be proportional to the number of viable cells in the well.

Quantification of FMCA results

Cell survival is presented as survival index (SI), which was defined as the fluorescence in experimental wells expressed as a percentage of that in the control wells. IC_{50} was defined as the concentration giving a SI 50% of the control SI.

Flow cytometric analysis of apoptosis and cell cycle distribution

Apoptosis was identified and quantified by flow cytometry with propidium iodide (PI; Sigma) staining [29]. Cells were treated with different concentrations of drugs for 72 h. At the end of the incubation, all floating and adherent cells were collected and fixed in 70% ice-cold ethanol, and kept in the freezer (–20°C) at least overnight until analysis. Fixed cells were washed twice with PBS and treated with 1 mg/ml RNAse (DNAse-free) for 30 min at 37°C. Cellular DNA was stained with 50 µg/ml PI in PBS containing 0.05% v/v Nonidet P-40. The cells were stored at 4°C overnight. Samples were filtered through a 30 µm pore size nylon mesh before

analysis by flow cytometry. At least 10,000 cells were collected for each sample. From the analysis of DNA histograms, the percentage of cells in different cycle phases was evaluated. Cells with a DNA content less than the cells in the G_1 phase (sub-G_1) were taken as apoptotic cells [9].

DNA fragmentation assay

The detection of apoptotic DNA fragments was performed as described previously [17]. Following incubation with As_2O_3 or As_2S_3 for 72 h, approximately 3×10^6 adherent and nonadherent cells were harvested after centrifugation. The harvested cells were washed twice with PBS, and then resuspended in 0.5 ml lysis buffer (1% NP-40, 50 m*M* Tris-HCl, pH 7.5, 20 m*M* EDTA). They were kept on ice for 10 min, mixed gently, and centrifuged in a microcentrifuge for 10 min at 14,000 *g* at 4°C, and the supernatant was saved. The extraction was repeated once. The supernatants were combined, and SDS and RNase A were added to a final concentration of 1% and 0.5 mg/ml, respectively. Samples were incubated at 56°C for 2 h, proteinase K was added to a final concentration of 2 mg/ml, and the mixture was incubated at 37°C for 2 h. One-half volume of 10 *M* ammonium acetate and 2.5 volumes of cold ethanol were added to the supernatant followed by incubation overnight at –20°C. DNA was collected by centrifugation at 14,000 *g* for 20 min at 4°C, and the DNA pellet was dissolved in 50 µl TE buffer (10 m*M* Tris-HCl, pH 7.5, 1 m*M* EDTA, pH 8.0). The DNA samples were separated by electrophoresis in a 2% agarose gel containing 0.5 µg/ml ethidium bromide in TAE buffer (40 m*M* Tris-acetate, pH 8.0, 2 m*M* EDTA). The gels were then visualized and photographed under UV light. Each experiment was repeated at least two times to confirm the results.

Statistics

Most experiments were repeated three times. In the FMCA experiments, all measurements were done in six replicates and the results are expressed as means ± SD for the data combined from separate experiments. The significance of differences between groups was determined by one-way ANOVA with the statistics software SPSS (Version 9.0.1, SPSS, Chicago, Ill.).

Results

Effect of As_2O_3 and As_2S_3 on cell morphology

After exposure to 2 µ*M* As_2O_3 or 5 µ*M* As_2S_3 for 72 h, the growth of CI80-13S, OVCAR and HeLa cells was clearly inhibited and there was a marked decrease in cell numbers compared to the control (Fig. 1). Typical apoptotic features were noted in CI80-13S, OVCAR and HeLa cells, including blebbing of the plasma membrane, chromatin condensation and formation of apoptotic bodies following exposure to As_2O_3 (2 µ*M*) or As_2S_3 (5 µ*M*). However, no significant changes in morphology or cell numbers were observed in GG or JAM cells or HF after the same treatment (Fig. 1).

As_2O_3 and As_2S_3 cause a dose-dependent inhibition of growth in ovarian cancer cell lines and cervical HeLa cells

To investigate the effects of As_2O_3 and As_2S_3 on cell survival and proliferation, four human ovarian cancer cell lines CI80-13S, OVCAR, GG and JAM, in addition to HeLa cells and HF were tested. These cell lines had different sensitivities to cisplatin as shown in Fig. 2. The dose-response curves of As_2O_3 and As_2S_3 for each cell line are shown in Fig. 3. Treatment of each cell line with different concentrations of As_2O_3 (0.1–20 µ*M*) or As_2S_3 (1–50 µ*M*) for 72 h led to a marked dose-dependent growth inhibition as determined in the FMCA. However, these cell lines exhibited distinctly different sensitivities to As_2O_3 and As_2S_3. CI80-13S, OVCAR and HeLa cells were very sensitive to As_2O_3 and As_2S_3: a significant reduction in cell survival was observed at very low concentrations of these arsenic compounds. The IC_{50} values of As_2O_3 and As_2S_3 in the cells exhibiting distinct sensitivities are shown in Table 1. The order of sensitivity to As_2O_3 was OVCAR≥HeLa≥CI80-13S > GG > JAM > HF. The cisplatin-resistant CI80-13S and the cisplatin-sensitive OVCAR ovarian cell lines and the cisplatin-sensitive cervical cell line HeLa were all very sensitive to As_2O_3. The IC_{50} values of As_2O_3 in these cell lines were about 2 µ*M* which was much lower than that in HF (10 µ*M*). These three cell lines were also more sensitive to As_2S_3 than HF. The IC_{50} values of As_2S_3 in these cell lines (5.61, 8.05 and 8.69 µ*M* for CI80-13S, OVCAR and HeLa, respectively) were much lower than in HF (44.29 µ*M*). The order of sensitivity to As_2S_3 was CI80-13S > OVCAR≥HeLa > GG > JAM > HF. In addition, all cancer cell lines tested showed significantly higher sensitivity to the two arsenic compounds than HF ($P < 0.05$).

As_2O_3 and As_2S_3 induce apoptosis in both cisplatin-resistant CI80-13S and cisplatin-sensitive OVCAR cells and cervical HeLa cells

To investigate whether the inhibition of cell proliferation by As_2O_3 and As_2S_3 was due to cell cycle arrest and/or apoptosis, cell cycle analyses were performed by flow cytometry. After treatment with 1, 2 and 3 µ*M* As_2O_3 or 1, 5 and 10 µ*M* As_2S_3 for 72 h, CI80-13S, OVCAR and HeLa cells exhibited a dose-dependent decrease in the percentage of cells in G_1 phase and a concomitant increase in the percentage of cells with less than G_1 (sub-G_1) phase compared to the untreated cells. However, in JAM and GG cells and HF after the same treatment there was no difference observed in the percentages of cells with a G_1 and sub-G_1 content of DNA compared to the control.

A distinct well-quantifiable region below the G_1 phase is a typical profile of apoptotic cells. DNA content distribution histograms clearly indicated that exposure of CI80-13S, OVCAR and HeLa cells to 2 µ*M* As_2O_3, a clinically achievable concentration, or 5 µ*M* As_2S_3 for 72 h resulted in the appearance of cells with a fractional DNA content forming a well-defined sub-G_1 peak. However, at the same concentrations, no major variation was observed in the HF, or GG and JAM cells. Figure 4 shows a representative DNA histogram for

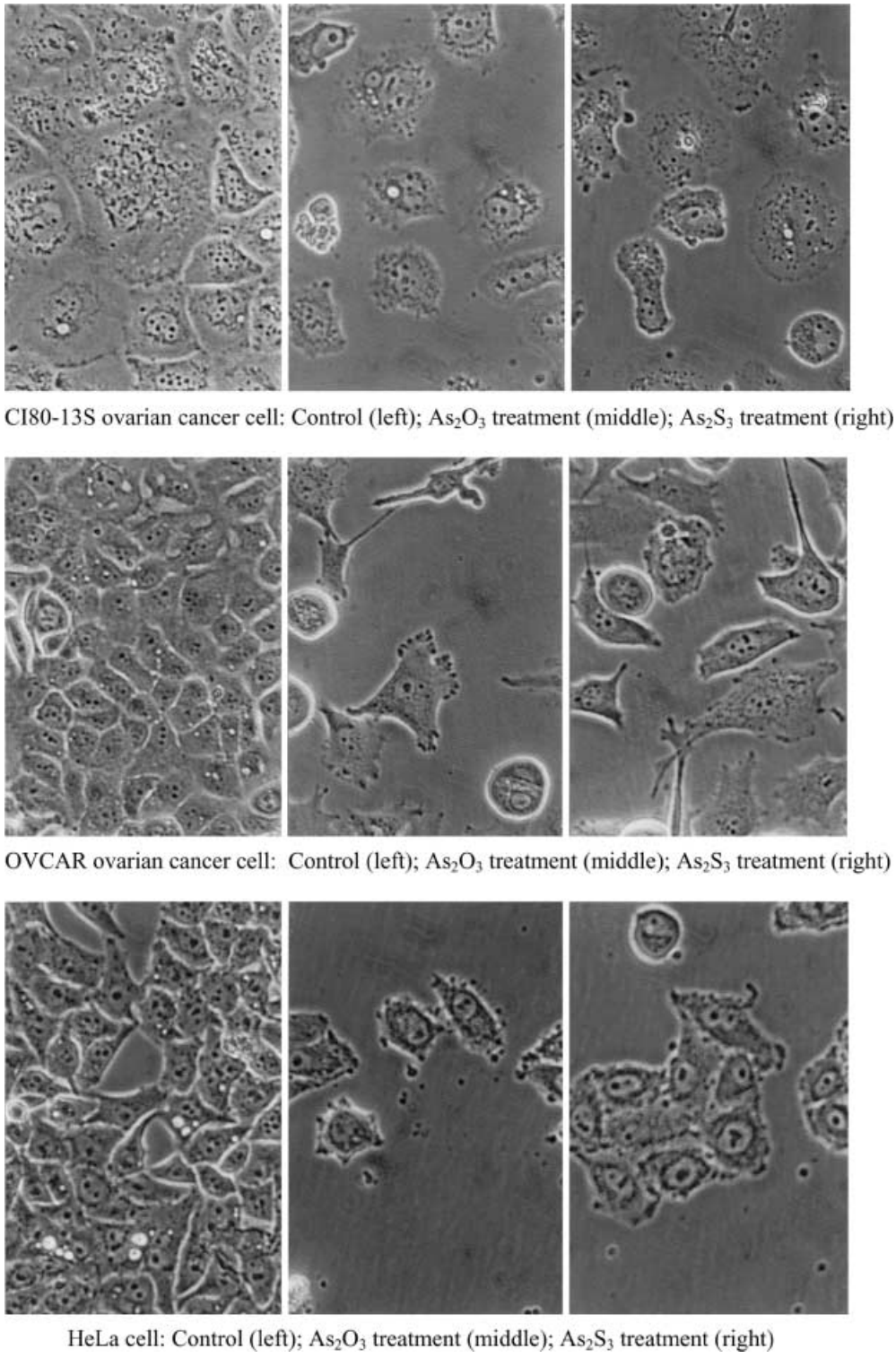


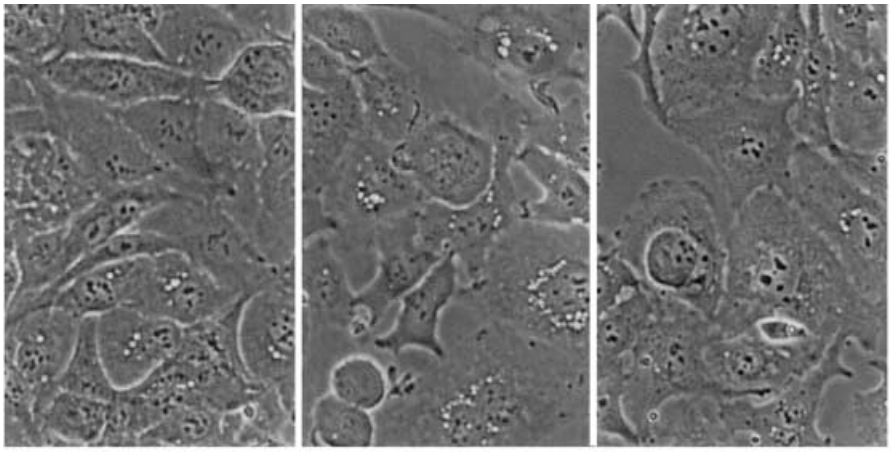
Fig. 1 Effect of As_2O_3 and As_2S_3 on cell morphology. Cells were treated with or without As_2O_3 (2 μ*M*) or As_2S_3 (5 μ*M*). After 72 h, the medium containing floating cells was removed and photographs of the adherent cells were taken under a phase-contrast microscope. Original magnification ×400

CI80-13S and GG cells and HF after treatment with 2 μ*M* As_2O_3 or 5 μ*M* As_2S_3 for 72 h. The percentages of apoptotic cells after treatment with As_2O_3 or As_2S_3 are shown in Fig. 5. At 2 μ*M* As_2O_3, the mean percentages of apoptotic cells were found to be 45.6%, 41.9% and 34.66%, respectively, in CI80-13S, OVCAR and HeLa cells, while the percentages of apoptotic cells in the GG and JAM cells and HF were all lower than 10%.

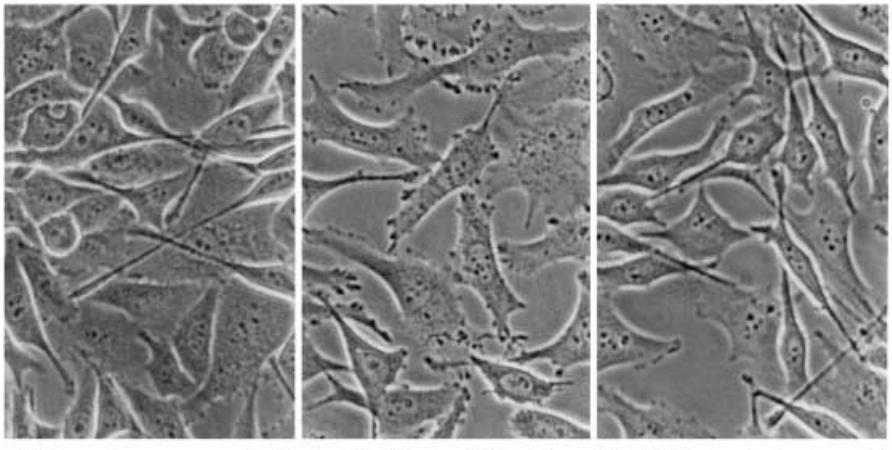
As_2O_3 and As_2S_3 induce DNA fragmentation in CI80-13S, OVCAR and HeLa cells

Internucleosomal DNA degradation is a very specific event in apoptosis. To further confirm that As_2O_3- or As_2S_3-treated cells demonstrated the biochemical characteristics of apoptosis, DNA degradation analysis was performed. The DNA from CI80-13S, OVCAR

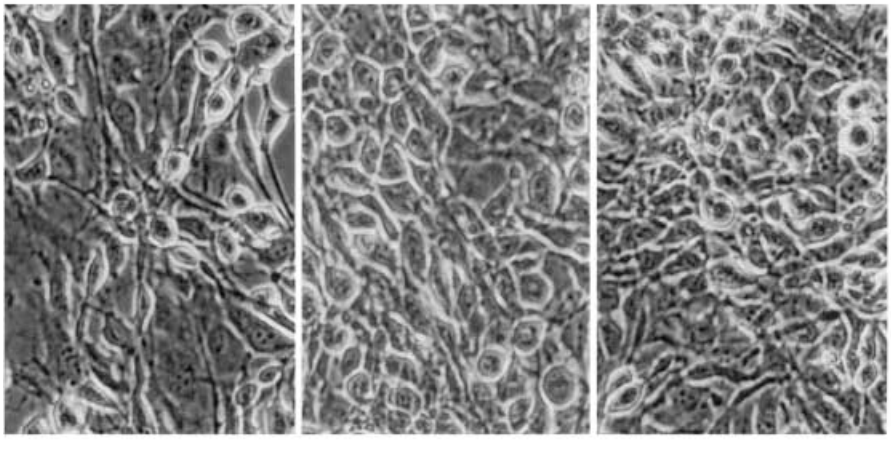
Fig. 1 Continued



GG ovarian cancer cell: Control (left); As_2O_3 treatment (middle); As_2S_3 treatment (right)



JAM ovarian cancer cell: Control (left); As_2O_3 treatment (middle); As_2S_3 treatment (right)



Human Fibroblast cell: Control (left); As_2O_3 treatment (middle); As_2S_3 treatment (right)

and HeLa cells displayed the characteristic internucleosomal ladder of DNA fragments after treatment with 2 μM As_2O_3 (Fig. 6A) and 5 μM As_2S_3 (Fig. 6B) for 72 h. In contrast, GG and JAM cells and HF did not show any DNA fragmentation after treatment with the same concentrations of As_2O_3 and As_2S_3 (data not shown).

Discussion

Apoptosis (programmed cell death) has emerged as an important biological mechanism that contributes to the maintenance of the integrity of multicellular organisms. It has been shown that tumors develop not only from

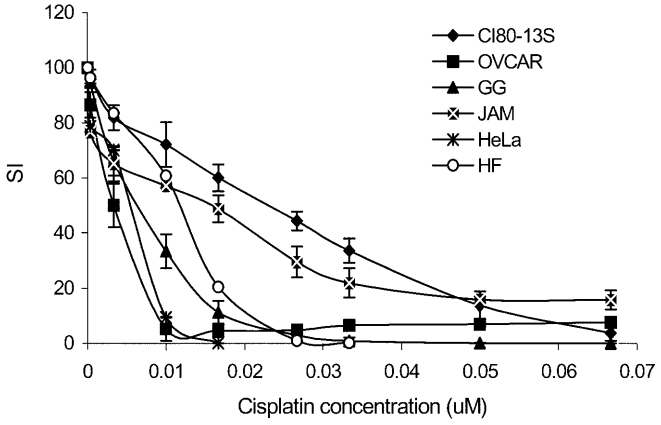


Fig. 2 The relative sensitivity of the ovarian cancer cell lines, cervical cancer cell lines and HF to cisplatin. The cells (10,000 per well) were incubated with increasing concentrations of cisplatin for 3 days and subsequently analyzed in the FMCA. The results are presented as the means ± SD from at least two individual experiments

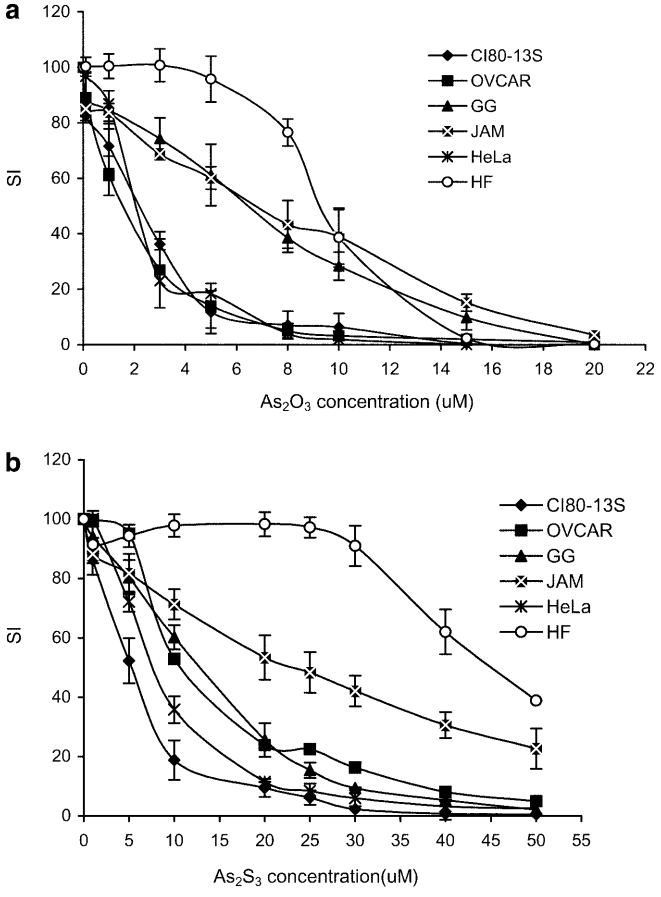


Fig. 3a, b The cytotoxic effects of As_2O_3 (**a**) and As_2S_3 (**b**) on the four human ovarian cancer cell lines, cervical HeLa cell line and HF. The cells (10,000 per well) were treated with the indicated concentrations of As_2O_3 or As_2S_3 for 3 days and then analyzed in the FMCA. The results are presented as means ± SD from at least three experiments. In each experiment, the data were calculated as the means from six replicates

abnormal cell proliferation and inhibition of differentiation, but also from reduced cell death due to inhibition of apoptosis [3]. Evidence suggests that the failure of cells to undergo apoptosis might be a factor in the pathogenesis of a variety of human diseases, including cancer, autoimmune diseases, and viral infections [32, 39]. In addition, a large number of diseases characterized by cell loss, such as neurodegenerative disorders, AIDS, and osteoporosis, may result from accelerated rates of physiological cell death. Cells from a wide variety of human malignancies have been shown to have a decreased ability to undergo apoptosis in response to at least some physiologic stimuli [18]. Apoptosis has become a focus of interest in oncology [13, 38]. Specific therapies are being designed to enhance the susceptibility of individual cell types from a variety of human cancers to undergo apoptosis for.

Recent studies have shown that As_2O_3 can induce apoptosis in the APL cell line NB4. Clinically, it is effective in the treatment of APL [34], and even in relapsed cases after all-*trans*-retinoic acid-induced remission [34]. Furthermore, As_2O_3, sodium arsenite [27] and melarsoprol, an organic arsenical [40], have also been shown to induce apoptosis in NB4 cells. However, the toxic effects of melarsoprol have restricted its clinical use. As_2S_3 is commonly used in traditional Chinese remedies and its tumorigenicity has been considered lower than that of other arsenic compounds such as calcium arsenate [31, 41].

In this study, we investigated the effect of As_2O_3 and As_2S_3 on cisplatin-sensitive and -resistant human ovarian and cervical cancer cell lines. Although cisplatin is effective in the treatment of ovarian cancer and cervical cancers, one of the major obstacles that limits its effectiveness is the frequent development of drug resistance during therapy. Treatment of these cell lines with different concentrations of As_2O_3 and As_2S_3 for 72 h led to a marked dose-dependent decrease in cell growth. The IC_{50} values for all the cancer cell lines examined were significantly lower than for HF. However, at 1–2 μM (clinically achievable concentrations that do not cause severe side effects [34]) As_2O_3 inhibited the growth of CI80-13S, OVCAR and HeLa cells. As_2S_3 at 5 μM also inhibited the growth of CI80-13S, OVCAR and HeLa cells. In contrast, the same concentrations of As_2O_3 and As_2S_3 had no significant growth inhibitory effect on GG or JAM cells or HF, suggesting that the effect of As_2O_3 and As_2S_3 was lineage restricted. They selectively inhibited both the cisplatin-resistant and -sensitive ovarian and cervical cancer cells, but had little effect on normal cells.

As_2O_3-induced apoptosis in APL cells is directly related to the downregulation of bcl-2 protein and modulation of PML-RARα fusion protein specific for APL cells with the t(15;17) translocation [5] and/or modulation of PML protein [46]. The activation of caspases is also involved in As_2O_3-induced apoptosis in APL cells [36]. It has been reported that at concentrations of 0.5–2.0 μM, As_2O_3 is able to trigger cell death of NB4 cells

Table 1 IC_{50} of As_2O_3 and As_2S_3 in different cell lines exposed for 3 days. The values are the averages from at least two separate experiments

	CI80-13S	OVCAR	GG	JAM	HeLa	HF
Cisplatin (μg/ml)	7.74 ± 1.7	1 ± 0.18	2.05 ± 0.89	5.53 ± 1.27	1.42 ± 0.01	3.1 ± 0.35
As_2O_3 (μ*M*)	2.1 ± 0.28*	1.88 ± 0.49*	6.32 ± 1.17*	7.26 ± 1.12*	1.85 ± 0.54*	10.02 ± 0.37
As_2S_3 (μ*M*)	5.61 ± 0.53*	8.05 ± 1.47*	12.73 ± 1.76*	22.99 ± 4.25*	8.69 ± 2.57*	44.29 ± 5.09

*$P < 0.05$ vs HF

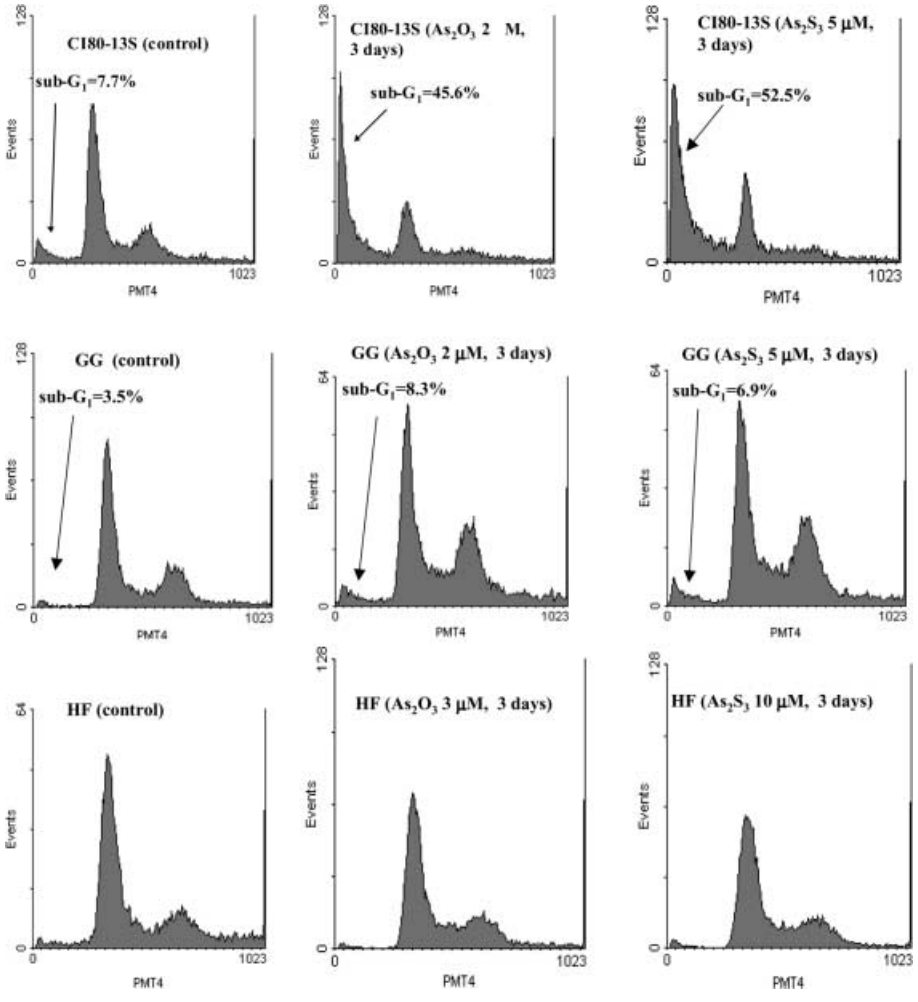


Fig. 4 Representative histogram of the cell cycle stage distribution of the ovarian cancer cells CI80-13S and GG and HF. The cells were treated with the indicated concentration of As_2O_3 or As_2S_3 for 3 days, stained with PI, and analyzed by flow cytometry

by apoptosis [5]. The growth inhibition of the cisplatin-resistant ovarian cancer cells CI80-13S, the cisplatin sensitive ovarian cancer cells OVCAR and cervical HeLa by As_2O_3 and As_2S_3 could be due to induction of apoptosis. This was confirmed by phase-contrast microscopy, flow cytometry and the DNA fragmentation assay.

The mechanisms involved in the relative arsenic sensitivity in different cell lines are not yet clear. Probably they are related to the biological properties and phenotype of the cells, the cellular metabolism of the arsenic compounds and/or different targets of action. Although the possible cellular mechanisms underlying the effects of arsenic treatment have been studied, the precise mechanism of action is still unknown. Zhang et al. [43] found that an increase in the intracellular concentration of Ca^{2+} is associated with As_2O_3-induced apoptosis in human gastric cancer MGC-803 and that a critical intracellular Ca^{2+} signal transduction pathway is involved in As_2O_3-mediated cell death. The sensitivity to As_2O_3-induced apoptosis in several cell lines including NB4 cells is inversely correlated with the intracellular glutathione content, which determines the sensitivity of cancer cells toward As_2O_3 [8]. In the study reported here we demonstrated that As_2O_3 and As_2S_3 were able to induce apoptosis in both cisplatin-resistant and -sensitive

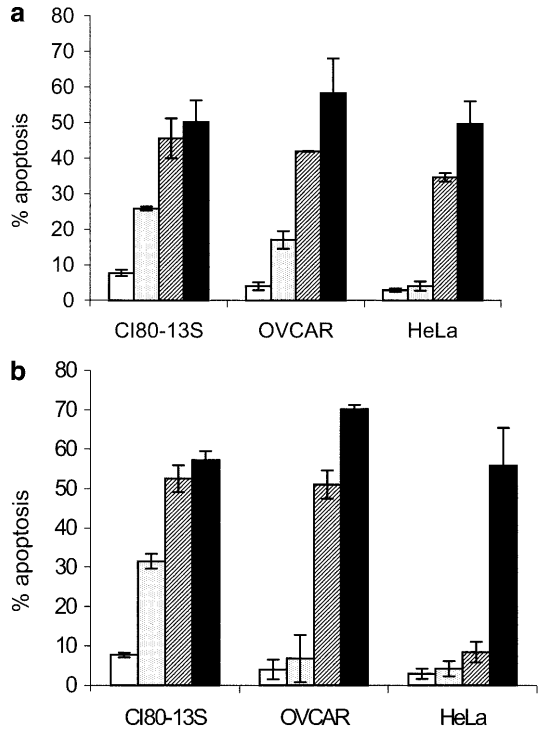
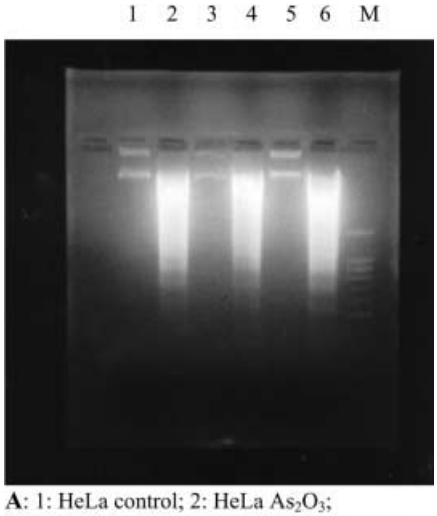
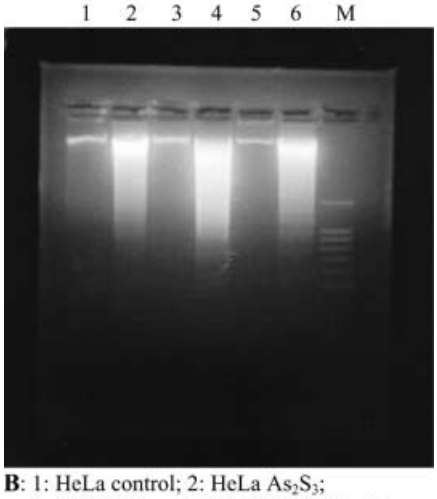


Fig. 5a, b Induction of apoptotic cell death by As_2O_3 and As_2S_3 in CI80-13S and OVCAR ovarian cancer cells and cervical HeLa cells. The cells were treated with 1, 2 and 3 μM As_2O_3 (**a**) or 1, 5 and 10 μM As_2S_3 (**b**). After 72 h of treatment, the floating and adherent cells were harvested, stained with PI and analyzed by flow cytometry. The percentage of apoptotic cells are indicated as the proportion of cells that contained $subG_1$ DNA. The means ± SD from at least three independent determinations are shown



A: 1: HeLa control; 2: HeLa As_2O_3; 3: CI80-13S control; 4 CI80-13S As_2O_3; 5: OVCAR control; 6: OVCAR As_2O_3; M: DNA marker



B: 1: HeLa control; 2: HeLa As_2S_3; 3: CI80-13S control; 4 CI80-13S As_2S_3; 5: OVCAR control; 6: OVCAR As_2S_3; M: DNA marker

Fig. 6A, B Induction of DNA fragmentation by As_2O_3 and As_2S_3. Cells were treated for 72 h with either vehicle (*control*) or 3 μM As_2O_3 (**A**) or 5 μM As_2S_3 (**B**), followed by lysis and extraction of soluble DNA. DNA samples were separated by electrophoresis in a 2% agarose gel containing 0.5 μg/ml ethidium bromide at 90 V for 45 min. The gels were visualized under UV light. The experiments shown are representative of three independent determinations with similar results

ovarian and cervical cancer cells. The high resistance to cisplatin developed by human ovarian cancer cell lines has been associated with marked increases in cellular glutathione synthesis [15]. Thus glutathione may not be involved in the selectivity of As_2O_3- and As_2S_3-induced apoptosis in the cisplatin-resistant and -sensitive ovarian and cervical cancer cells. The mechanisms of action of As_2O_3 and As_2S_3 in these cells lines needs further investigation.

Many anticancer drugs exert their action via apoptosis. The selective induction of apoptosis in tumor cells without affecting the healthy cells would give fewer side effects of treatment. This has been the major goal in the development of chemotherapeutic agents. We demonstrated that low concentrations of As_2O_3 and As_2S_3 were able to selectively inhibit the growth of ovarian and cervical cancer cells while having little effect on the normal HF. The findings not only provide preliminary evidence that As_2O_3 and As_2S_3 could inhibit human ovarian and cervical cancer cells in vitro, but also demonstrated that at clinically achievable concentrations of these compounds the effects were selective and specific. Our findings suggest that As_2O_3 and As_2S_3 might be promising agents in the treatment of ovarian and cervical cancer sensitive or resistant to cisplatin.

References

1. Akao Y, Mizoguchi H, Kojima S, Naoe T, Ohishi N, Yagi K (1998) Arsenic induces apoptosis in B-cell leukaemic cell lines in vitro: activation of caspase and down-regulation of Bcl-2 protein. Br J Haematol 102(4):1055
2. Akao Y, Nakagawa Y, Akiyama K (1999) Arsenic trioxide induces apoptosis in neuroblastoma cell lines through the activation of caspase 3 in vitro. FEBS Lett 455(1-2):59

3. Bergamaschi G, Rosti V, Danova M, Lucotti C, Cazzola M (1994) Apoptosis: biological and clinical aspects. Haematologica 79:86
4. Byrd DM, Roegner ML, Griffiths JC, Lamm SH, Grumski KS, Wilson R, Lai S (1996) Carcinogenic risks of inorganic arsenic in perspective. Int Arch Occup Environ Health 68:484
5. Chen GQ, Zhu J, Shi XG, Ni JH, Zhong HJ, Si GY, Jin XL, Tang W, Li XS, Xong SM, Shen ZX, Sun GL, Ma J, Zhang P, Zhang TD, Gazin C, Naoe T, Chen SJ, Wang ZY, Chen Z (1996) In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB4 cell apoptosis with down-regulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins. Blood 88(3):1052
6. Chen GQ, Shi XG, Tang W, Xiong SM, Zhu J, Cai X, Han ZG, Ni JH, Shi GY, Jia PM, Liu MM, He KL, Niu C, Ma J, Zhang P, Zhang TD, Paul P, Naoe T, Kitamura K, Miller W, Waxman S, Wang ZY, deThe H, Chen SJ, Chen Z (1997) Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): I. As_2O_3 exerts dose-dependent dual effects on APL cells. Blood 89(9):3345
7. Chen Z, Wang ZY, Chen SJ (1997) Acute promyelocytic leukemia: cellular and molecular basis of differentiation and apoptosis. Pharmacol Ther 76:141
8. Dai J, Weinberg RS, Waxman S, Jing YK (1999) Malignant cells can be sensitized to undergo growth inhibition and apoptosis by arsenic trioxide through modulation of the glutathione redox system. Blood 93:268–277
9. Darzynkiewicz Z, Bruno S, Del Bino G, Gorczyca W, Hotz MA, Lassota P, Traganos F (1992) Features of apoptotic cells measured by flow cytometry. Cytometry 13(8):795
10. Dive C, Hickman JA (1991) Drug-target interactions: only the first step in the commitment to a programmed cell death? Br J Cancer 64:192
11. Dong Y, Berners-Price SJ, Thorburn DR, Antalis T, Dickinson J, Harst T, Qiu L, Khoo SK, Parsoms PG (1997) Serine protease inhibition and mitochondrial dysfunction associated with cisplatin resistance in human tumor cell line: targets for therapy. Biochem Pharmacol 53:1673
12. Eliopoulos AG, Kerr DJ, Herod J, Hodgkins L, Krajewski S, Reed JC, Young LS (1995) The control of apoptosis and drug resistance in ovarian cancer: influence of p53 and Bcl-2. Oncogene 11(7):1217
13. Fisher DE (1994) Apoptosis in cancer therapy: crossing the threshold. Cell 78:539
14. Forkner C, McNair-Scott TF (1931) Arsenic as a therapeutic agent in chronic myeloid leukemia. JAMA 97:305
15. Godwin AK, Meister A, O'Dwyer PJ, Huang CS, Hamilton TC, Anderson ME (1992) High resistance to cisplatin in human ovarian cancer cell lines is associated with marked increase of glutathione synthesis. Proc Natl Acad Sci USA 89:3070
16. Harris CC (1996) Structure and function of the p53 tumor suppressor gene: clues for rational cancer therapeutic strategies. J Natl Cancer Inst 88:1442
17. Herrmann M (1994) A rapid and simple method for the isolation of apoptotic DNA fragments. Nucleic Acids Res 22(24):5506
18. Hoffman B, Liebermann DA (1994) Molecular controls of apoptosis: differentiation/growth arrest primary response genes, proto-oncogenes, and tumor suppressor genes as positive and negative modulators. Oncogene 9(7):1807
19. Huang SL, Guo AX, Xiang Y (1995) Clinical study on the treatment of APL mainly with composite Indigo Naturalis tablets. Clin J Hematol 16:26
20. Huang XJ, Wiernik PH, Klein RS, Gallagher RE (1999) Arsenic trioxide induces apoptosis of myeloid leukemia cells by activation of caspases. Med Oncol 16(1):58
21. Johnson SW, Laub PB, Beesley JS, Ozols RF, Hamilton TC (1997) Increased platinum-DNA damage tolerance is associated with cisplatin resistance and cross-resistance to various chemotherapeutic agents in unrelated human ovarian cancer cell lines. Cancer Res 57(5):850
22. Larsson R, Nygren P (1989) A semiautomated fluorometric method for determination of cytotoxicity and cellular proliferation of human tumor cell lines in microculture. Anticancer Res 9:1111–1120
23. Larsson R, Kristensen J, Sandberg C, Nygren P (1992) Laboratory determination of chemotherapeutic drug resistance in tumor cells from patients with leukemia using a fluorometric microculture cytotoxicity assay (FMCA). Int J Cancer 50:177
24. Lehmann S, Bengtzen S, Paul C (1997) Arsenic trioxide induces apoptosis and cytotoxic effects in blast cells from patients with non-M3 AML. Blood 90 [Suppl. 1]:325a
25. Look AT (1998) Arsenic and apoptosis in the treatment of acute promyelocytic leukemia. J Natl Cancer Inst 90:86
26. Lu M, Levin J, Sulpice E, Sequeira-Le Grand A, Alemany M, Caen JP, Han ZC (1999) Effect of arsenic trioxide on viability, proliferation, and apoptosis in human megakaryocytic leukemia cell lines. Exp Hematol 27:845
27. Ma DC, Sun YH, Chang KZ, Ma XF, Huang SL, Bai YH, Kang J, Liu YG, Chu JJ (1998) Selective induction of apoptosis of NB4 cells from G2 + M phase by sodium arsenite at lower doses. Eur J Haematol 61:27
28. Moreland NJ, Illand M, Kim YT, Paul J, Brown R (1999) Modulation of drug resistance mediated by loss of mismatch repair by the DNA polymerase inhibitor aphidicolin. Cancer Res 59(9):2102
29. Nicoletti I, Migliorati G, Pagliacci MC, Grignani F, Riccardi C (1991) A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. J Immunol Methods 139(2):271
30. Perkins C, Kim CN, Fang G, Bhalla KN (2000) Arsenic induces apoptosis of multidrug-resistant human myeloid leukemia cells that express Bcr-Abl or overexpress MDR, MRP, Bcl-2, or Bcl-x(L). Blood 95(3):1014
31. Pershagen G, Bjorklund NE (1985) On the pulmonary tumorigenicity of arsenic trisulfide and calcium arsenate in hamsters. Cancer Lett 27(1):99
32. Rew DA (1998) Cell and molecular mechanisms of pathogenesis and treatment of cancer. Postgrad Med J 74:77
33. Shao W, Fanelli M, Ferrara FF, Riccioni R, Rosenauer A, Davison K, Lamph WW, Waxman S, Pelicci PG, Lo Coco F, Avvisati G, Testa U, Peschle C, Gambacorti-Passerini C, Nervi C, Miller WH Jr (1998) Arsenic trioxide as an inducer of apoptosis and loss of PML/RAR alpha protein in acute promyelocytic leukemia cells [see comments]. J Natl Cancer Inst 90:124
34. 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(9):3354
35. Shen ZY, Shen J, Cai WJ, Hong C, Zheng MH (2000) The alteration of mitochondria is an early event of arsenic trioxide induced apoptosis in esophageal carcinoma cells. Int J Mol Med 5(2):155
36. Soignet SL, Maslak P, Wang ZG, Jhanwar S, Calleja E, Dardashti LJ, Corso D, DeBlasio A, Gabrilove J, Scheinberg DA, Pandolfi PP, Warrell RP (1998) Complete remission after treatment of acute promyelocytic leukemia with arsenic trioxide. New Engl J Med 339:1341–1348
37. Sun HD, Ma L, Hu XC, Zhang TD (1992) Treatment of acute promyelocytic leukemia (31 cases) with intravenous arsenic trioxide. Chin J Integr Chin West Med 12:170
38. Thompson CB (1995) Apoptosis in the pathogenesis and treatment of disease. Science 267:1456
39. Vaux DL, Haecker G, Strasser A (1994) An evolutionary perspective on apoptosis. Cell 76(5):777
40. Wang ZG, Rivi R, Delva L, Konig A, Scheinberg DA, Gambacorti-Passerini C, Gabrilove JL, Warrell RP Jr, Pandolfi PP (1998) Arsenic trioxide and melarsoprol induce programmed cell death in myeloid leukemia cell lines and function in a PML and PML-RARalpha independent manner. Blood 92(5):1497

41. Yamamoto A, Hisanaga A, Ishinishi N (1987) Tumorigenicity of inorganic arsenic compounds following intratracheal instillations to the lungs of hamsters. Int J Cancer 40(2):220
42. Zhang P, Wang SY, Hu LH, Shi FD, Qiu FD, Hong RJ, Han XY (1996) Arsenic trioxide treated 72 cases of acute promyelocytic leukemia. Chin J Hematol 17:58
43. Zhang TC, Cao EH, Li JF, Ma W, Qin JF (1999) Induction of apoptosis and inhibition of human gastric cancer MGC-803 cell growth by arsenic trioxide. Eur J Cancer 35(9):1258
44. Zhang W, Ohnishi K, Shigeno K, Fujisawa S, Naito K, Nakamura S, Takeshita K, Takeshita A, Ohno R (1998) The induction of apoptosis and cell cycle arrest by arsenic trioxide in lymphoid neoplasms. Leukemia 12(9):1383
45. Zheng J, Deng YP, Lin C, Fu M, Xiao PG, Wu M (1999) Arsenic trioxide induces apoptosis of HPV16 DNA-immortalized human cervical epithelial cells and selectively inhibits viral gene expression. Int J Cancer 82:286
46. Zhu J, Koken MHM, Quignon F, ChelbiAlix MK, Degos L, Wang ZY, Chen Z, deThe H (1997) Arsenic-induced PML targeting onto nuclear bodies: implications for the treatment of acute promyelocytic leukemia. Proc Natl Acad Sci USA 94:3978
47. Zhu XH, Shen YL, Jing YK, Cai X, Jia PM, Huang Y, Tang W, Shi GY, Sun YP, Dai J, Wang ZY, Chen SJ, Zhang TD, Waxman S, Chen Z, Chen GQ (1999) Apoptosis and growth inhibition in malignant lymphocytes after treatment with arsenic trioxide at clinically achievable concentrations. J Natl Cancer Inst 91(9):772