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ORIGINAL ARTICLE

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Arsenic trioxide induces apoptosis equally in T lymphoblastoid leukemia MOLT-4 cells and P-gp-expressing daunorubicin-resistant MOLT-4 cells

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Abstract *Purpose*: To investigate the effects of arsenic trioxide (As_2O_3) on human T-lymphoblastoid leukemia MOLT-4 cells and P-gp-expressing daunorubicin-resistant MOLT-4 (MOLT-4/DNR) cells. *Methods*: Cell growth was measured by an MTT assay. Cell viability was determined by a dye exclusion test. The level of P-gp expression was estimated using phycoerythrin-conjugated anti-P-gp monoclonal antibody 17F9. The function of P-gp was evaluated in terms of rhodamine 123 (Rh123) efflux. The percentage of cells undergoing apoptosis was determined by flow cytometry after staining with annexin V-FITC and propidium iodide. *Results*: As_2O_3 inhibited the growth and survival of MOLT-4 and MOLT-4/DNR cells in a time- and dose-dependent manner. The 50% inhibitory concentrations of As_2O_3 (IC_{50}) against the growth of these cell lines were 5.1 μmol/l and 5.0 μmol/l, respectively, when the cells were treated with As_2O_3 for 96 h. As_2O_3 induced an apoptotic morphology in both MOLT-4 and MOLT-4/DNR cell lines. These effects of As_2O_3 were time- and dose-dependent when the two cell lines were incubated in the presence of 1–8 μmol/l of As_2O_3 for 3–144 h. As_2O_3 treatment for 3 to 24 h at 5.0 μmol/l did not change the percentage of P-gp-expressing cells or the efflux ability of MOLT-4/DNR cells. *Conclusion*: As_2O_3 inhibited growth and induced apoptosis equally in MOLT-4 and MOLT-4/DNR cells, and this suppressive effect did not influence P-gp expression or function in MOLT-4/DNR cells.

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Introduction

Arsenic trioxide (As_2O_3) has recently been confirmed to be an effective treatment for acute promyelocytic leukemia (APL) both in patients with newly diagnosed APL and in those with refractory and relapsed APL [17, 18, 27, 32, 33, 34, 37]. The effects of As_2O_3 have been shown not only in parental APL NB4 cells [7], but also in retinoic acid-resistant APL cells [3]. It has been reported that the effects of As_2O_3 are not confined to APL cells but can also be observed in various other cell lines of myeloid [35] and lymphoid origin [31, 38, 39] and in drug-resistant sublines [13, 29]. The mechanisms of action of As_2O_3 in APL and other malignancies are thought to involve inhibition of growth and induction of apoptosis [1, 3, 7, 38, 39].

MOLT-4, a human T-lymphoblastoid leukemia cell line, has been used extensively for studies of leukemia cell biology and antileukemia therapy [2, 11]. We have established a daunorubicin-resistant MOLT-4 subline (MOLT-4/DNR) by exposing the parental MOLT-4 cells stepwise to increasing concentrations of DNR over 3 months [23]. These resistant MOLT-4/DNR cells have been shown to overexpress functional P-glycoprotein (P-gp) and MDR1 mRNA [23].

P-gp, a product of the multidrug resistance (MDR) gene, is a transmembrane efflux pump for different lipophilic compounds, including many anticancer drugs and fluorescent dyes [5]. Intracellular drug accumulation in cells expressing functional P-gp has been found to be decreased as a result of drug efflux [12]. Rhodamine 123 (Rh123) accumulates in the mitochondria of cells and is used as a standard functional indicator of MDR [4, 24]. The function of P-gp can also be evaluated by inhibition of Rh123 efflux with MDR modulators [25]. Cyclosporin A (CsA) has been found to decrease Rh123 efflux from

MDR-expressing cells [16]. Most of the drugs excreted via this efflux pump are hydrophobic organic compounds, and As_2O_3 may not be excluded from drug-resistant cell lines expressing functional P-gp. However, whether As_2O_3 affects the growth of lymphocytic leukemia cells expressing functional P-gp, or the expression and/or function of P-gp, is unknown.

Thus, in the present study, we investigated the effects of As_2O_3 on the growth of and apoptosis in parental MOLT-4 and resistant MOLT-4/DNR cells, and we discuss here the possible mechanisms of growth suppression by As_2O_3 in these cell lines.

Materials and methods

Reagents

RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, N.Y.). Cell proliferation kits I and II (MTT) were purchased from Roche Diagnostics (Indianapolis, Ind.). Trypan blue, Rh123, DNR and As_2O_3 were obtained from Sigma Chemical Company (St. Louis, Mo.). DNR stock solutions were made at a concentration of 10 mmol/l with ethanol and diluted to working concentrations before use. As_2O_3 stock solutions were made at a concentration of 5 mmol/l with phosphate-buffered saline (PBS) and diluted to working concentrations before use. Mouse anti-human P-gp monoclonal antibody conjugated with R-phycoerythrin (R-PE) (monoclonal antibody 17F9) and R-PE-conjugated mouse IgG2b isotype control monoclonal antibody were obtained from PharMingen (San Diego, Calif.). The annexin V-FITC apoptosis detection kit I was obtained from BD PharMingen.

Cell culture

MOLT-4 and MOLT-4/DNR cells were maintained in RPMI-1640 medium containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin as described previously [14, 15]. The leukemia cells were washed and resuspended with the above medium to 5×10^5 cells/ml, then 196 μl of the cell suspension was placed in each well of a 96-well flat-bottomed plate. To the suspension in each well was added 4 μl PBS solution containing As_2O_3 and 4 μl ethanol solution containing DNR to yield final concentrations of 0.25, 0.5, 1, 2, 4, 5, 6, 8 and 16 μmol/l and 0.001, 0.01, 0.1, 1 and 10 μmol/l, respectively; 4 μl PBS and 4 μl ethanol were added to the control wells, respectively. The cells were incubated for 96 h in an atmosphere of air containing 5% CO_2 at 37°C in a humidified chamber.

MTT assay

After the incubation period, 10 μl of MTT labeling reagent (final concentration 0.5 mg/ml) was added to each well and the plate was placed on a microshaker for 10 s, after which the cells were further incubated for 4 h in a humidified atmosphere. Subsequently, 100 μl of solubilization solution (Roche) was added to each well and the plate was placed on a microshaker for 10 s, after which the plate was left overnight in an incubator in a humidified atmosphere. The spectrophotometric absorbance of the samples was measured on a microplate reader (Corona MT P-32; Corona Company, Japan) at 570 nm. A dose response curve was plotted for each drug, and the concentrations that yielded a 50% inhibition of cell growth (IC_{50}) were calculated.

Viability of MOLT-4 and MOLT-4/DNR cells

MOLT-4 and MOLT-4/DNR cell lines were cultured by initially seeding 2×10^5 cells/ml of fresh RPMI-1640 medium containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin in the presence of various concentrations of As_2O_3 for 24 to 144 h in an atmosphere of air containing 5% CO_2 at 37°C in a humidified chamber. To avoid any possible effects of cell density on cell growth and survival, cells were maintained at less than 5×10^5 cells/ml by daily adjusting the cell concentration by adding fresh culture medium and the corresponding concentrations of As_2O_3 when necessary. The number or percentage of viable cells was determined by staining the cell populations with trypan blue. Before carrying out the experiments, four parts of 0.2% trypan blue (w/v in water) were mixed with one part of saline (4.25% NaCl w/v in water), and one part of the trypan blue saline solution was added to one part of the cell suspension. The cells were then loaded into a hemocytometer and the number of unstained (viable) cells and stained (dead) cells were counted separately within 3 min of staining with trypan blue. Actual cell numbers were calculated by multiplying the initial cell numbers by the times dilution.

Morphological changes in As_2O_3-treated MOLT-4 and MOLT-4/DNR cells

MOLT-4 and MOLT-4/DNR cells suspended at 5×10^5 cells/ml in fresh medium were cultured in the presence or absence of As_2O_3 for 24 to 144 h in an atmosphere of air containing 5% CO_2 at 37°C in a humidified chamber. At the indicated times, pictures were taken under a microscope (Olympus Optical Company, Japan).

P-gp expression

MOLT-4 and MOLT-4/DNR cells suspended at 1×10^6 cells/ml in fresh medium were cultured in the presence or absence of As_2O_3 for 3 to 24 h in an atmosphere of air containing 5% CO_2 at 37°C in a humidified chamber. At the indicated times, the cells were collected and washed twice in washing buffer (PBS/0.1% NaN_3, pH 7.2). Cells were resuspended in 50 μl buffer and then incubated with either 20 μl of monoclonal antibody 17F9 or 20 μl of the R-PE-conjugated mouse IgG2b isotype control monoclonal antibody for 30 min in the dark. The cells were then centrifuged again in washing buffer (PBS/0.1% NaN_3, pH 7.2) at 1300 rpm for 5 min at 4°C. The cells were resuspended in 400 μl staining buffer (PBS/0.1% NaN_3/1% FBS, pH 7.2) and P-gp expression was determined by flow cytometry (Becton Dickinson). The data were analyzed with Cell Quest software (Becton Dickinson).

P-gp efflux function

Cells (1×10^6) were collected and centrifuged at 1300 rpm for 5 min at 4°C, resuspended in buffer containing 5 μg/ml of Rh123, and left to stand for 10 min in an atmosphere of air containing 5% CO_2 at 37°C. After washing, the cells were incubated in the presence or absence of As_2O_3 or inhibitor (CsA) for the indicated times in an atmosphere of air containing 5% CO_2 at 37°C. After incubation, the cells were washed twice in washing buffer and resuspended in 400 μl staining buffer. The remaining intracellular Rh123 fluorescence intensity was determined by a flow cytometry (Becton Dickinson). The data were analyzed with Cell Quest software (Becton Dickinson).

Apoptosis assays

MOLT-4 and MOLT-4/DNR cells (1×10^6/ml) were treated with the indicated concentrations of As_2O_3. After the indicated incubation times, cells were washed twice in cold PBS (pH 7.2) and resuspended in binding buffer at a density of 1×10^6 cells/ml, and then 1×10^5 cells were stained with 5 μl annexin V-FITC and 5 μl propidium iodide (Becton Dickinson) for 15 min at room temperature in the dark. Then 400 μl binding buffer was added to each tube,

and the cells were analyzed by flow cytometry (Becton Dickinson) within 1 h of staining. A total of 30,000 non-gated cells were analyzed.

Statistics

Comparison of the data between two groups was carried out using Student's *t*-test. Comparison of the data among multiple (more than three) groups was carried out using the Bonferroni-Dun multiple comparison. In each case, *P* values less than 0.05 were considered significant.

Results

Effects of As_2O_3 on parental MOLT-4 and resistant MOLT-4/DNR cell growth

MOLT-4 and MOLT-4/DNR cells were continuously treated with As_2O_3 at concentrations in the range 0.25 to 16.0 μmol/l for 96 h, and cell growth was measured in an MTT assay. The growth of MOLT-4 and MOLT-4/DNR cells was suppressed by As_2O_3 treatment in a dose-dependent manner. The IC_{50} values in the parental and subline cells were 5.1 μmol/l and 5.0 μmol/l, respectively (Fig. 1a).

At the same time, the viability of MOLT-4 and MOLT-4/DNR cells similarly decreased when the cells were incubated in the presence of As_2O_3 at a concentration of 5 μmol/l for 24 to 144 h in a time-dependent manner (Fig. 2a). Following treatment with As_2O_3 for 4 days at concentrations of 1 to 16 μmol/l, the viability of cells of both lines decreased to a similar extent and in a dose-dependent manner (Fig. 2b).

The effects of DNR on the growth of MOLT-4 and MOLT-4/DNR cells were examined after 96 h in culture. The IC_{50} value of DNR in MOLT-4 cells assessed by the MTT assay was 0.1 μmol/l, and in MOLT-4/DNR cells was 12 times higher (1.2 μmol/l) (Fig. 1b). Thus, DNR was effective against parental MOLT-4 cells, but less effective against MOLT-4/DNR cells, as expected.

Additive effect of As_2O_3 with DNR on MOLT-4/DNR cell growth

As described above, MOLT-4/DNR cells were less sensitive to DNR alone at concentrations of 0.001 to 0.1 μmol/l, but when MOLT-4/DNR cells were cultured in the presence of DNR combined with As_2O_3 at 2 μmol/l, their growth was inhibited (Fig. 3a). The suppressive effects of As_2O_3 and DNR appeared to be additive, and depended on the dose of DNR (Fig. 3a). As described above, the effects of As_2O_3 on the growth of MOLT-4/DNR cells were observed at As_2O_3 concentrations >2 μmol/l (Fig. 1a, Fig. 2b). However, when the cells were treated with As_2O_3 at concentrations in the range 0.25 to 2 μmol/l combined with 0.1 μmol/l DNR, the effect of As_2O_3 on cell growth was additively increased (Fig. 3b).

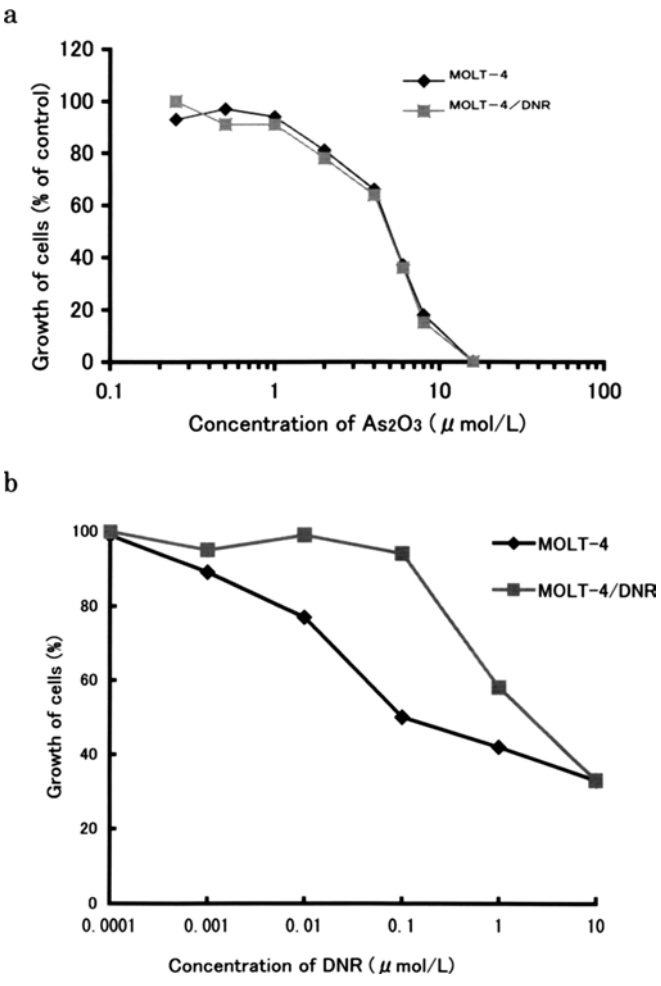


Fig. 1a, b Effects of As_2O_3 (**a**) and DNR (**b**) on in vitro growth of MOLT-4 and MOLT-4/DNR cells. Cells were treated with different concentrations of As_2O_3 and DNR for 4 days. Cell growth was determined by an MTT assay. Values are the means of three independent experiments

As_2O_3 did not change the P-gp expression and function of MOLT-4/DNR cells

Staining of cells with FITC-conjugated mouse anti-human MDR monoclonal antibody revealed that only 2.5% of MOLT-4 cells expressed P-gp, whereas 93.7% of MOLT-4/DNR cells expressed P-gp (Fig. 4a). To evaluate the P-gp efflux function, 5 μg/ml Rh123 as a P-gp substrate and 100 ng/ml CsA as an MDR modulator were used in the P-gp efflux function experiments. MOLT-4/DNR cells incubated for 3 h in the absence of CsA showed a rapid decrease in intercellular Rh123 levels from $87.1 \pm 0.6\%$ to $53.3 \pm 8.8\%$ ($P < 0.01$). The efflux was inhibited by CsA, and the intracellular Rh123 levels increased again to $76.6 \pm 1.2\%$ ($P < 0.05$) in the presence of CsA. In contrast, parental MOLT-4 cells still retained the dye (from $80.4 \pm 3.6\%$ to $80.8 \pm 7.8\%$). This result showed that MOLT-4/DNR cells possess a high level of P-gp efflux activity (Fig. 4b).

When MOLT-4/DNR cells were cultured in the presence of As_2O_3 at a concentration of 5 μmol/l for 3 to

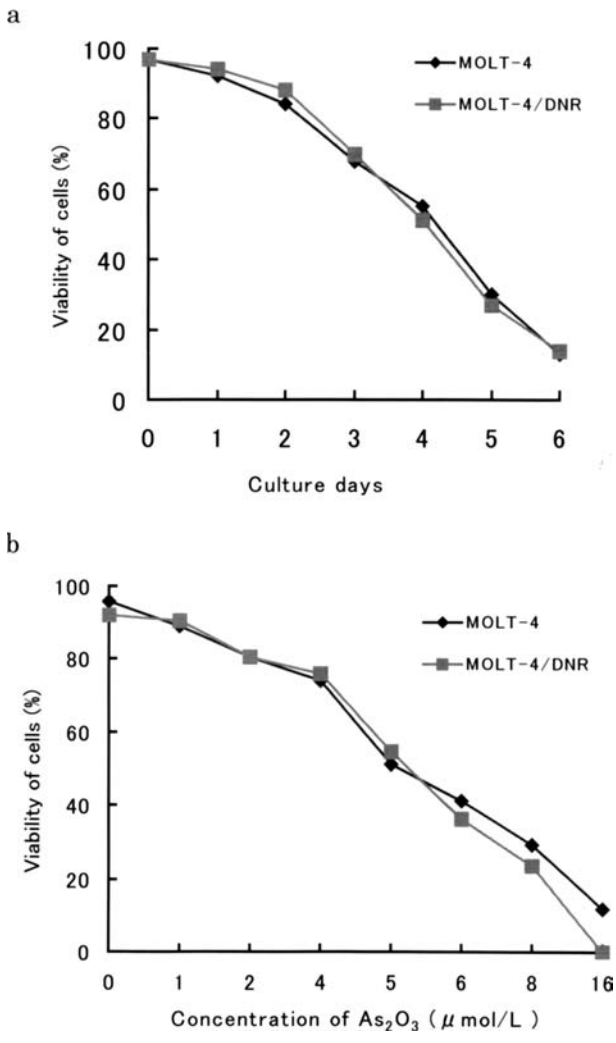


Fig. 2a, b Comparison of viabilities of cells treated with As_2O_3 as a function of culture days (**a**) and concentration of As_2O_3 (**b**) between MOLT-4 and MOLT-4/DNR cells (**a** cells treated with 5 μmol/l of As_2O_3, **b** cells treated with the agent for 4 days). Cell viability was determined by a dye exclusion test. Values are the means of three independent experiments

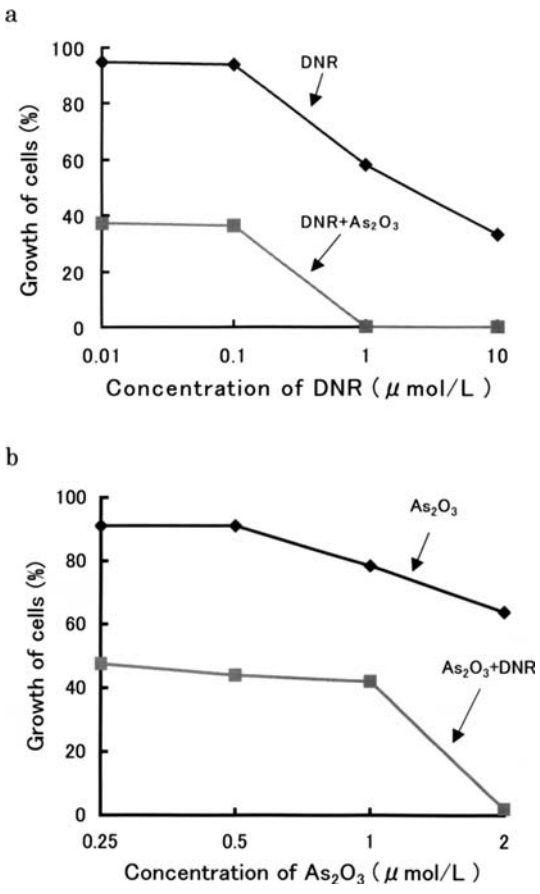


Fig. 3a, b Additive effect of DNR and As_2O_3 on the growth of MOLT-4/DNR cells (**a** DNR concentration varied, As_2O_3 concentration kept constant at 2 μmol/l; **b** As_2O_3 concentration varied, DNR concentration kept constant). Cells were treated for 4 days. Cell growth was determined by an MTT assay. Values are the means of three independent experiments

24 h, the percentages of P-gp expression did not change from those in the absence (0 h) of As_2O_3 (Fig. 5). When MOLT-4/DNR cells were incubated with As_2O_3 for 3, 6 or 24 h at concentrations of 1 to 8 μmol/l, the percentage of cells expressing P-gp also did not change as compared to control.

In MOLT-4/DNR cells, intracellular Rh123 accumulation was significantly enhanced in the presence of CsA at a concentration of 100 ng/ml ($P < 0.05$, Fig. 6), but was not significantly changed in MOLT-4/DNR cells cultured in the presence of As_2O_3 at 5 μmol/l ($P > 0.05$. Fig. 6).

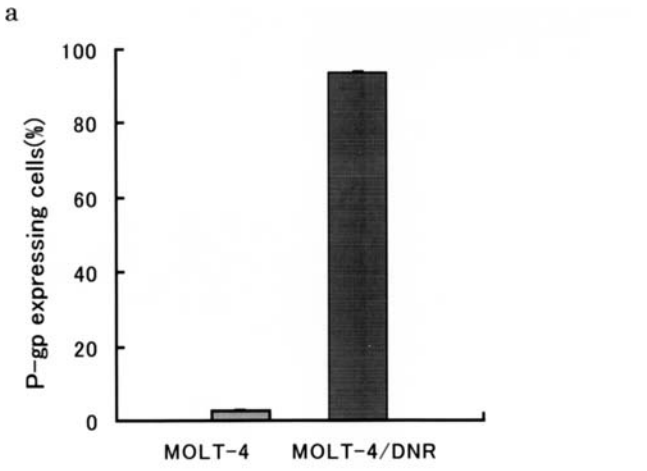
As_2O_3 induces apoptosis in cells of both the MOLT-4 and MOLT-4/DNR cell line

MOLT-4 and MOLT-4/DNR cells showed a similar round morphology after culture for 96 h (Fig. 7a, b), but after treatment with various concentrations of As_2O_3 for 96 h cells of both lines exhibited the typical morphological characteristics of apoptosis including chromatin condensation and fragmentation of nuclei (Fig. 7c, d). Numerous apoptotic bodies, which are membrane-enclosed vesicles that have budded off cytoplasmic extensions, were also observed in these cells (Fig. 7c, d).

In MOLT-4 and MOLT-4/DNR cells cultured in the presence of As_2O_3 for 4 days at concentrations of 1 to 8 μmol/l, the percentage of apoptotic cells as determined by annexin V increased in a dose-dependent manner (Fig. 8a), and when these cells were incubated with As_2O_3 at a concentration of 5 μmol/l for 3 to 144 h, the percentage of apoptotic cells increased in a time-dependent manner (Fig. 8b).

Discussion

The results described above showed that As_2O_3 inhibited growth and induced apoptosis equally in MOLT-4 cells and MOLT-4/DNR cells. The results also suggested that the effects of As_2O_3 on growth of MOLT-4/DNR cells



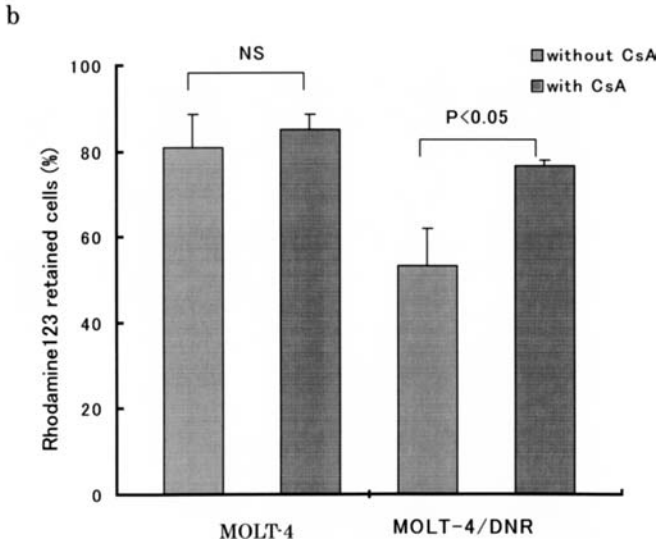


Fig. 4a, b Comparison of P-gp expression (**a**) and function (**b**) between MOLT-4 and MOLT-4/DNR cells. **b** Effects of incubation for 3 h with the inhibitor CsA at 100 ng/ml on the P-gp efflux function of MOLT-4 and MOLT-4/DNR cells. P-gp expression and function were determined by flow cytometry as described in Materials and methods. Values are the means ± SD of three independent experiments

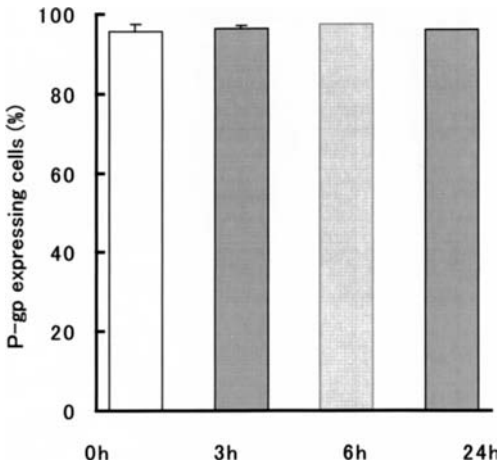


Fig. 5 Effect of As_2O_3 on P-gp expression of MOLT-4/DNR cells. Cells were incubated in the presence of 5 μmol/l As_2O_3 for 3, 6 and 24 h, and the percentages of cells expressing P-gp determined by flow cytometry. Values are the means ± SD of three independent experiments

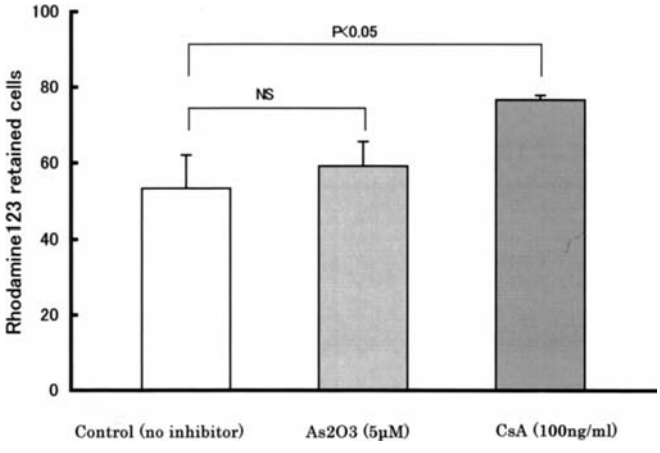


Fig. 6 Comparison of As_2O_3 and CsA in inhibiting P-gp efflux function. P-gp efflux function was determined by flow cytometry as described in Materials and methods. Values are the means ± SD of three independent experiments

were not due to suppression of P-gp expression or function in these cells.

The MOLT-4/DNR cell line was derived in our laboratory from the parental MOLT-4 cell line by exposing the cells stepwise to increasing concentrations of DNR [23], and the cell line was shown to be persistently resistant to DNR. The IC_{50} value of DNR in MOLT-4/DNR cells was 12 times higher than that in parental MOLT-4 cells as assessed by the MTT assay. The resistance of MOLT-4/DNR cells to DNR has been reported to be closely correlated with the expression of functional P-gp [23].

MDR is recognized as one of the most common causes of failure of chemotherapy in the treatment of cancer patients [19, 20, 21]. P-gp is an ABC (ATP-binding cassette) transporter, which hydrolyses ATP and expels cytotoxic drugs from mammalian cells [30]. The fluorescent dye Rh123 has been found to be transported by P-gp [9, 26], and the flow cytometric measurement of cellular Rh123 uptake/efflux is an efficient tool to assess the functional activity of P-gp in tumor samples [25]. In this work, we revealed that 94% of MOLT-4/DNR cells express P-gp, and the percentage of P-gp expression in MOLT-4/DNR cells was significantly higher than that in MOLT-4 cells (less than 3%). Furthermore, MOLT-4/DNR cells exhibited significantly higher P-gp efflux activity than parental MOLT-4 cells. However, both parental MOLT-4 and resistant MOLT-4/DNR cell lines were highly sensitive to As_2O_3. As_2O_3 similarly inhibited growth and induced apoptosis in these two cell lines. These effects of As_2O_3 were time- and dose-dependent. These results suggest that P-gp in MOLT-4/DNR cells is not involved in the detoxification of As_2O_3.

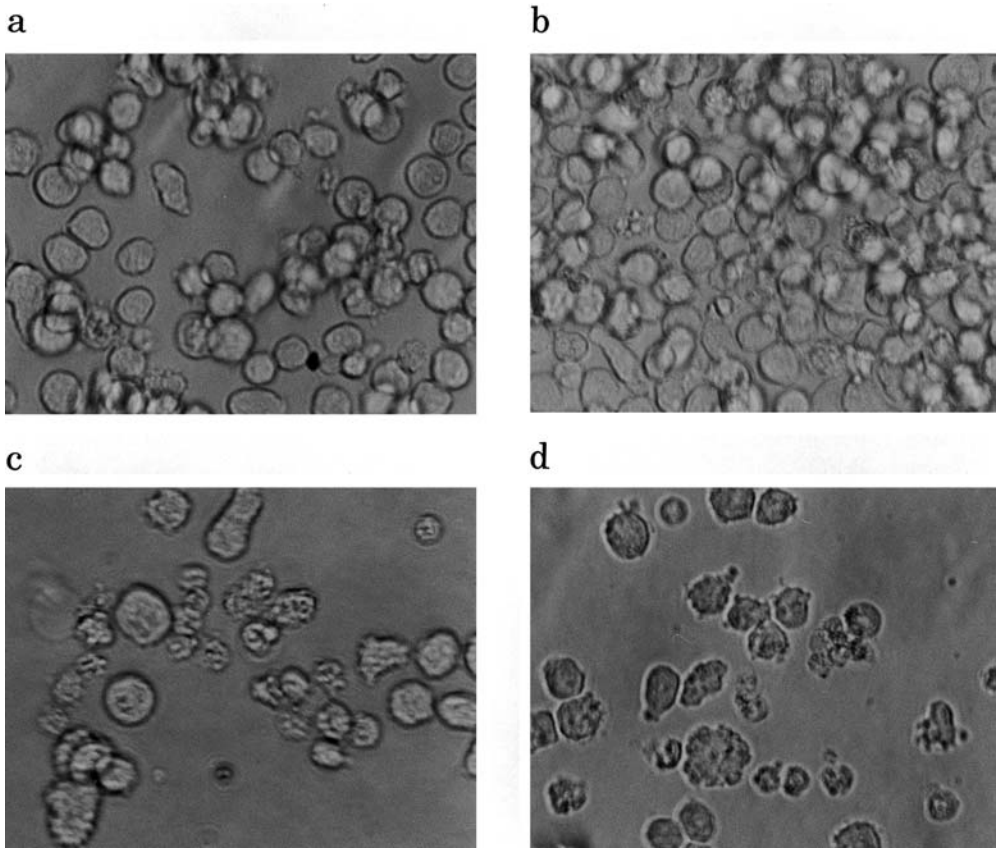


Fig. 7a–d Cell morphology after treatment with 5 μmol/l As_2O_3 for 4 days. **a** Untreated MOLT-4 cells, **b** untreated MOLT-4/DNR cells, **c** As_2O_3-treated MOLT-4 cells, **d** As_2O_3-treated MOLT-4/DNR cells

Indeed, the expression of P-gp in resistant MOLT-4/DNR cells did not revert in the presence of As_2O_3 at concentrations of 1 to 8 μmol/l, which were efficiently cytotoxic for the cell line. The efflux function of P-gp in MOLT-4/DNR cells also did not change in the presence of various concentrations of As_2O_3. These observations confirm our conclusion that As_2O_3 exhibited cytotoxicity without influencing functional P-gp in the resistant MOLT-4/DNR cells.

Arsenic is a natural substance that has been used medicinally for over 2400 years [36]. Recent clinical studies in China have shown that As_2O_3 is an effective and relatively safe drug in the treatment of APL [17, 18, 32, 37]. As_2O_3 can inhibit growth and trigger apoptosis in cells of the APL cell line NB4 [6, 7]. In particular, As_2O_3 is also effective in APL patients who are resistant to all-*trans*-retinoic acid (ATRA) and conventional chemotherapy [3, 6]. In some in vitro studies, the growth and survival of ATRA-resistant APL subline cells have been found to be inhibited effectively by As_2O_3 [3, 7]. In addition, the suppressive effect of As_2O_3 is not specific for APL cells, but can be observed in various cell lines of myeloid [35], lymphoid [31, 38, 39] or T-cell origin [10, 22]. Among multiple myeloma cells, the P-gp-positive cell line 8226/Dox40 undergoes apoptosis in response to As_2O_3 in vitro, in a similar manner to the drug-sensitive cell line 8226/S [13]. Otherwise, As_2O_3-induced apoptosis in HL-60/VCR and HL-60/AR cells is not significantly different from that in HL-60/neo cells [29]. Thus, previous results suggest that As_2O_3 is not a substrate for the mdr1 gene-encoded P-gp. In this study, we demonstrated that As_2O_3 is not sensitive to drug efflux pump mechanisms of resistance.

Studies on clinical pharmacokinetics of As_2O_3 have shown that the peak level of plasma As_2O_3 concentration under a general treatment schedule is 5.5 to 7.3 μmol/l [32]. In the present study, the concentrations of As_2O_3 inducing apoptosis in vitro were from 1 to 8 μmol/l in a dose- and time-dependent manner in both MOLT-4 and MOLT-4/DNR cells. A remarkably effective concentration of As_2O_3 inducing apoptosis in these cell lines was 5 μmol/l, which is not over the peak level in clinical trials.

Previous studies based on experiments with the APL cell line NB4 have indicated that As_2O_3 causes apoptosis directly through downregulation of bcl-2 [6]. On the other hand, the glutathione (GSH) redox system is known to modulate the growth-inhibitory effect of arsenicals [8, 28]. It was found that the sensitivity to As_2O_3-induced apoptosis is inversely related to the intracellular GSH content and that pharmacological modulation of intracellular GSH contents influences sensitivity to As_2O_3 [8]. Thus, the effects of As_2O_3 in inhibiting growth and inducing apoptosis in the parental MOLT-4 and the resistant MOLT-4/DNR cells may possibly be related to the regulation of suppressor gene(s) particularly bcl-2 and the GSH redox system.

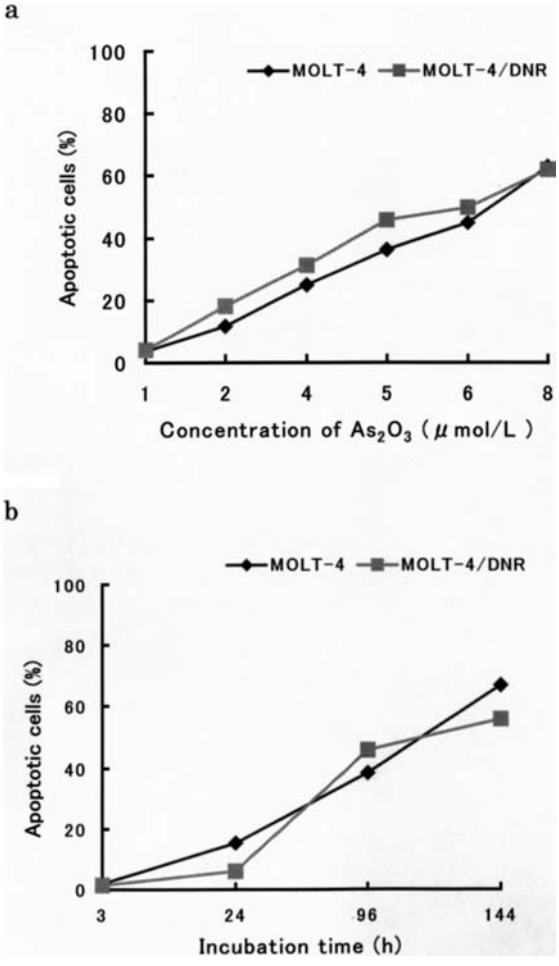


Fig. 8a, b Induction of apoptosis by As_2O_3 in MOLT-4 and MOLT-4/DNR cells. **a** Percent apoptotic cells as a function of As_2O_3 concentration after culture for 4 days, **b** Percent apoptotic cells as a function of incubation time in the presence of 5 μmol/l As_2O_3. Values are the means of three independent experiments

Since As_2O_3 showed no effect on P-gp expression and function in MOLT-4/DNR cells, the additive efficacy of As_2O_3 with DNR possibly results from the apoptosis-inducing effect of As_2O_3. This apoptosis-inducing effect of As_2O_3 is caused by regulation of bcl-2 and/or depletion of GSH from the leukemic cells [6, 8, 28]. DNR is known to block DNA replication and RNA synthesis, and therefore, the apoptosis-inducing effect of As_2O_3 is possibly additive to (or synergistic with) the pharmacological action of DNR against the growth of MOLT-4/DNR cells. While P-gp may, in part, be involved in the efflux of DNR out of the cells in the presence of As_2O_3 in MOLT-4/DNR cells, a combination of these drugs may work additively through different growth-suppressing mechanisms. This additive effect can cause a considerable shift in the dose-response curve of each agent. These findings may imply potential clinical uses of DNR in combination with As_2O_3 for the treatment of patients with T-lymphoblastoid leukemia exhibiting DNR resistance.

In conclusion, we showed in the present study that As_2O_3 was effective in suppressing growth and in inducing apoptosis in both the parental MOLT-4 cell line and its DNR-resistant subline expressing functional P-gp. Thus, the present findings may be relevant for further study of the clinical use of As_2O_3 against drug-resistant T lymphoblastoid leukemia.

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