Glutathione-*S*-transferase π inhibits As_2O_3-induced apoptosis in lymphoma cells: involvement of hydrogen peroxide catabolism

Li Zhou, Yongkui Jing, Miroslav Styblo, Zhu Chen, and Samuel Waxman

Arsenic trioxide (As_2O_3) is an effective agent for the treatment of relapsed and refractory acute promyelocytic leukemia by induction of partial differentiation and apoptosis. As_2O_3, at therapeutic concentrations (1-2 μM), induced apoptosis in Raji lymphoma cells but not in Jurkat lymphoma cells, which inversely correlated with the levels of glutathione-*S*-transferase π (*GSTP1*), but not $GSTM_1$ and $GSTA_1$, expression and activity. *GSTP1* mRNA, protein level, and activity were high in Jurkat cells but undetectable in Raji cells. Stable transfection of *GSTP1* into Raji cells decreased the amount of As_2O_3-induced apoptosis. Apoptosis induced by therapeutic concentrations of As_2O_3 in Raji cells is related to increasing H_2O_2 intracellular accumulation but not to JNK activation. Forced expression of *GSTP1* by transfection of Raji cells significantly decreased the basal amount of H_2O_2 and its levels after therapeutic concentration of As_2O_3 treatment. Added exogenous H_2O_2 was removed more rapidly, which correlated with a greater decrease in reduced glutathione level in Raji clones expressing *GSTP1* than in those clones without *GSTP1* expression. Overexpression of *GSTP1* in transfected Raji clones was also found to decrease the retention of As_2O_3. These data suggest that *GSTP1* blocks As_2O_3-induced apoptosis in lymphoma cells by decreasing intracellular amounts of H_2O_2 by catabolism and H_2O_2 production by decreasing the intracellular retention of As_2O_3. (Blood. 2005;105: 1198-1203)

Introduction

Arsenic trioxide (As_2O_3) is an effective agent for the treatment of relapsed and refractory acute promyelocytic leukemia (APL) and acts by inducing apoptosis and partial differentiation.[1-3] Although As_2O_3 induces apoptosis in other forms of malignant tumors, different mechanisms of action have been identified. In some leukemia and lymphoma cells, As_2O_3, at therapeutic concentrations (1-2 μM), induces apoptosis through radical oxygen species–mediated pathways.[4-8] Moreover, in leukemia cells, the H_2O_2 scavenging systems (glutathione peroxidase and catalase) and arsenic detoxification systems (glutathione-*S*-transferase and glutathione) are key factors in controlling cell sensitivity to As_2O_3-induced apoptosis.[4] However, in solid tumors, As_2O_3, at high concentrations (greater than 10 μM), is required to induce apoptosis that involves the Jun N-terminal kinase (JNK)–mediated pathway.[9-13]

Glutathione-*S*-transferase π (*GSTP1*), a member of the As_2O_3 detoxification pathway, is increased in several arsenic-resistant cell lines.[14-18] As_2O_3-sensitive NB4 cells have low levels of *GSTP1* compared with other leukemia cell lines.[4] These observations suggest that *GSTP1* may be directly involved in As_2O_3-induced apoptosis. Jurkat cells are less sensitive to arsenic-induced apoptosis than Raji cells,[19] and *GSTP1* is highly expressed in Jurkat cells but undetectable in Raji cells.[20] In the present report, the role of *GSTP1* in As_2O_3-induced apoptosis was studied in these 2 lymphoma cell lines. Raji cells were stably transfected with *GSTP1*, and the effects of *GSTP1* on apoptosis, H_2O_2 accumulation, intracellular accumulation of arsenic, and JNK activation were compared in *GSTP1*-expressing and nonexpressing cells. It was found here that the overexpression of *GSTP1* in Raji cells decreased the ability of therapeutic concentrations of As_2O_3 to induce apoptosis. As_2O_3-treated *GSTP1*-expressing Raji cells were also found to accumulate less intracellular H_2O_2 and As_2O_3 than Raji cells not expressing *GSTP1,* demonstrating 2 mechanisms for *GSTP1* mediation of As_2O_3-induced apoptosis.

Materials and methods

Reagents

Arsenic (III) oxide (As_2O_3, at least 99% pure) and anticatalase monoclonal antibody were purchased from Sigma (St Louis, MO); G418 sulfate was purchased from Fisher Scientific (Pittsburgh, PA); anti-GST P1-1, anti-GST M1-1, and anti-GST A1-1 polyclonal antibodies were purchased from Calbiochem (La Jolla, CA); and antiphospho-SAPK/JNK (Thr183/Tyr185) monoclonal antibody (anti–p-JNK), anti-SAPK/JNK polyclonal antibody, antiphospho-p38 mitogen-activated protein (MAP) kinase (Thr180/Thr182) polyclonal antibody, and anti-p38 MAP kinase polyclonal antibody were purchased from Cell Signaling Technology (Beverly, MA). Antiglutathione peroxidase was obtained from Abcam (Cambridge, MA). Carrier-free [^{73}As]-arsenite ($^{73}As^{V}$) was purchased from Los Alamos Meson Production Facility (Los Alamos, NM). [^{73}As]-arsenate ($^{73}As^{III}$) was prepared from $^{73}As^{V}$ by reduction with metabisulfite/thiosulfate reagent.[34] Yields of $^{73}As^{III}$

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in this reaction typically exceeded 95%, as determined by thin-layer chromatography (TLC).[21]

Cell lines and culture conditions

Raji and Jurkat human lymphoma cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and were cultured in RPMI 1640 medium adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES (*N*-2-hydroxyethylenepiperazine-*N*′-2-ethanesulfonic acid), 1.0 mM sodium pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA), and 10% fetal bovine serum (JRH BioScience, Lenexa, KS) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.

Stable transfection of *GSTP1*

Raji cells were transfected by electroporation with a pcDNA3.1 plasmid with or without a *GSTP1* expression sequence (kindly provided by Dr Ze'ev Ronai, Mount Sinai School of Medicine, New York). Briefly, 10^6 Raji cells in 1 mL mixed with 20 μg pcDNA3.1/GSTP1 plasmid were transferred to sterile electroporation cuvettes (Bio-Rad, Hercules, CA) and were electroporated in a GenePulser (Bio-Rad) with the voltage set at 300 V and the capacitor at 250 μF. After transfection, the cells were incubated in fresh medium containing 1 mg/mL G418 for 4 weeks. Subsequently, cell clones resistant to G418 were isolated and screened by limited dilution. Two *GSTP1*-expressing and 2 *GSTP1*-nonexpressing cell clones were selected for further study. Transfected Raji cell clones were routinely cultured in medium containing 1 mg/mL G418 and then were cultured for at least 24 hours without G418 before an experiment was initiated.

Viability assay

Cells were seeded at 1.5 to 2 × 10^5 cells/mL and were cultured in medium described with or without the indicated concentrations of test compounds for the times indicated. Cell viability was estimated by trypan blue dye exclusion, and cell numbers were determined by hemocytometer.

Apoptosis assay

Apoptotic cells were detected by Annexin V assay. In general, 10^6 cells were washed twice with phosphate-buffered saline (PBS), then labeled by Annexin V–fluorescein isothiocyanate (FITC) and propidium iodide (PI) in binding buffer according to the instructions in the Annexin V–FITC Apoptosis Detection Kit provided by the manufacturer (Oncogene, Cambridge, MA). Fluorescence signals of FITC and PI were detected by FL1 (FITC detector) at 518 nm and FL2 at 620 nm, respectively, on a FACScan (Becton Dickinson, San Jose, CA). The log of Annexin V–FITC fluorescence was displayed on the x-axis, and the log of PI fluorescence was displayed on the y-axis. Data were analyzed using the CELLQuest (Becton Dickinson) software. For each analysis, 10 000 events were recorded.

Intracellular H_2O_2 production

Intracellular H_2O_2 level was detected as previously reported by using 5,6-carboxy-2′,7′-dichlorofluorescein-diacetate (DCFH-DA; Molecular Probes, Eugene, OR).[4] Briefly, 2 hours before ending the indicated treatment, 5 μM DCFH-DA was added to the medium and was continuously incubated for 2 hours at 37°C; then the fluorescence intensity was measured by FACScan (Becton Dickinson).

GSTP1-1 activity assay

*GS*TP1-1 activity was measured as previously described using 1-chloro-2, 4-dinitrobenzene (CDNB) as a high-affinity substrate and compared with that of other GST isoforms.[22] Briefly, 5 × 10^7 cells were washed twice with cold PBS, resuspended in 300 μL of 100 mM potassium phosphate buffer (pH 6.8), and sonicated for 10 seconds at 4°C. After centrifugation at 17 000 *g* for 30 minutes, 50 μL cell lysate were mixed with 850 μL of 0.1 mM EDTA (ethylenediaminetetraacetic acid) (pH 6.5), 50 μL of 20 mM glutathione, and 50 μL of 20 mM CDNB. Absorbance of the mixture was continuously recorded for 2 minutes at 340 nm on a spectrophotometer (Ultrospec 2000; Pharmacia Biotec, Uppsala, Sweden).

Measurement of intracellular glutathione

Intracellular glutathione (GSH) contents were measured using the Glutathione Assay Kit (Calbiochem, San Diego, CA). In brief, 5 × 10^6 cells were homogenized in 5% metaphosphoric acid using a Teflon pestle (Racine, WI). Particulate matter was separated by centrifugation at 4000*g*. Supernatant was used for GSH measurement according to the manufacturer's instruction. The GSH content was expressed as nmol/10^6 cells.

Western blot analysis

Cells were centrifuged, washed with cold PBS, and lysed on ice for 30 minutes in RIPA buffer (1 × PBS, 1% nonidet P-40 [NP40], 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS]) containing protease and phosphatase inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 mM Na_3VO_4, 5 mM NaF, 1 × protease inhibitor [Boehringer Mannheim GmbH, Mannheim, Germany]). Protein concentrations were determined with a Bio-Rad protein assay kit (Bio-Rad). Twenty-five micrograms to 70 μg total protein was electrophoresed on 8% to 12% SDS polyacrylamide gels, then transferred to a nitrocellulose membrane (Amersham, Piscataway, NJ). After incubating with 5% nonfat milk (Nestle Carnation) for an hour, the membrane was incubated with the indicated primary antibody overnight at 4°C, washed with Tris-buffered saline (pH 6.8) with Tween-20 (TBS-T) 3 times, incubated with secondary antibody for 1 hour at room temperature, and washed with TBS-T 3 times. The membrane was analyzed by autoradiography using a chemiluminescence kit (Amersham Life Science, Buckinghamshire, United Kingdom).

Northern blot analysis

Total RNA was isolated from 10^6 cells with an RNA isolation kit (Gentra, Minneapolis, MN). Twenty micrograms RNA was size fractionated on a 1.2% agarose–2.2 M formaldehyde gel, transferred to hybrid-N+ membrane (Amersham) in 20 × standard sodium citrate (SSC) solution, and UV cross-linked (Stratalinker; Stratagene, La Jolla, CA). Whole-length complementary DNA (cDNA) of *GSTP1* was used as the probe. Probes were labeled with ^{32}P-dCTP by random priming to a specific activity of 0.5 to approximately 1 × 10^9 cpm/ng. Membranes were prehybridized for 4 hours at 42°C in 50% formamide, 6 × sodium chloride, sodium phosphate, EDTA (SSPE), 5 × Denhardt reagent, and 0.2 mg/mL salmon sperm DNA and then were hybridized with a radiolabeled probe. Membranes were washed twice in 6 × SSC containing 0.1% SDS, followed by a stringent wash with 0.2 × SSC containing 0.1% SDS at 65°C.

^{73}As retention assay

Cells (10^6) were incubated in complete medium with 1 μM ^{73}AsIII–carrier at 37°C in a humidified atmosphere containing 5% CO_2 for 24 hours. Cell were centrifuged at 500 rpm for 5 minutes and washed twice with PBS. Radioactivity in the sediment and in the medium plus washes was determined with the aid of a gamma counter.

Statistical analysis

Data were analyzed for statistical significance using the Student *t* test (Microsoft Excel; Microsoft, Seattle, WA). Differences were considered significant at *P* less than .05.

Results

As_2O_3 induces apoptosis in Raji cells, but not in Jurkat cells, at therapeutic concentrations

Apoptosis induction and growth inhibition abilities of As_2O_3 in Jurkat and Raji cells were compared after As_2O_3 treatment at therapeutic concentrations of 1 to 2 μM. As shown in Figure 1A,

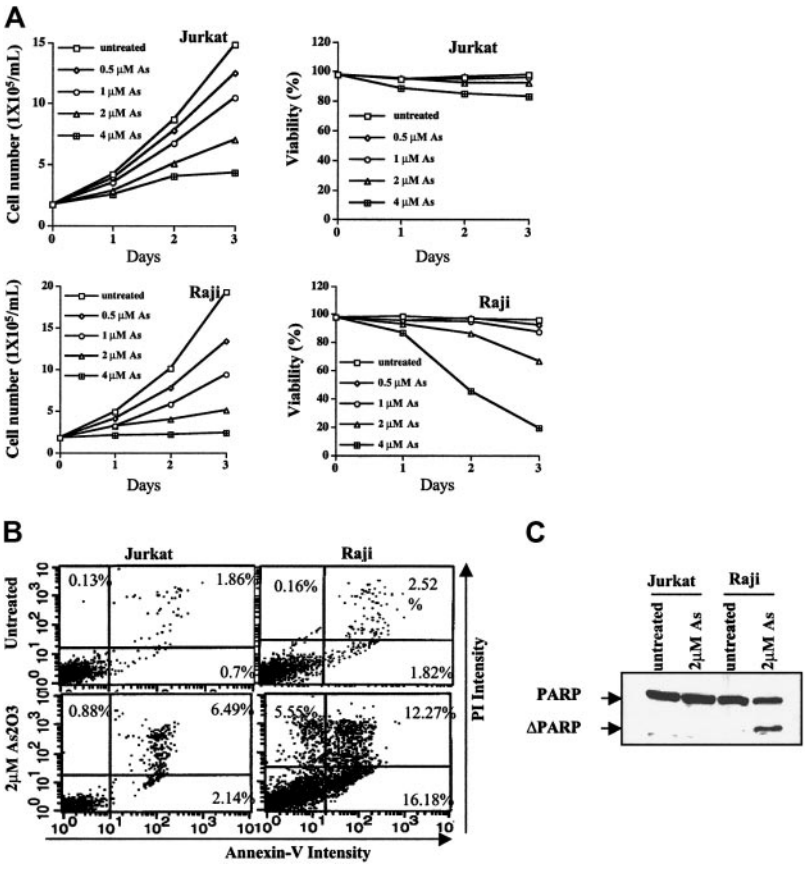

Figure 1. Growth inhibition and apoptosis induction by As_2O_3 in Jurkat and Raji cells. (A) Cell growth rate and viability. Jurkat and Raji cells were treated or untreated with the indicated concentrations of As_2O_3. Cell concentrations and percentages of viable cells after staining with trypan-blue were determined with the aid of a hematometer. Each value represents the mean ± SD of triplicates. (B) Apoptosis induction. Jurkat and Raji cells were untreated or treated with As_2O_3, 2 μM, for 3 days. Percentages of apoptotic cells were determined by staining with Annexin V and PI on flow cytometry, as described in "Materials and methods." (C) Western blot analysis of PARP cleavage. Cells were untreated or were treated with As_2O_3, 2 μM, for 3 days. Anti-PARP antibody was used to detect the PARP cleavage product, as described in "Materials and methods."

As_2O_3 treatment for 3 days caused more growth inhibition in Raji cells (inhibitory concentration 50% [IC_{50}], approximately 0.9 μM) than in Jurkat cells (IC_{50}, approximately 2 μM). In addition, the viability of Raji, but not of Jurkat, cells was decreased by As_2O_3 treatment in concentration- and time-dependent manners. As_2O_3 (2 μM) treatment for 3 days reduced the viability of Raji cells by 35%, whereas the viability of Jurkat cells was greater than 92% before and after As_2O_3 treatment. Percentages of apoptotic cells induced by 2 μM As_2O_3 in Raji and Jurkat cells was 34% and 9%, respectively. The extent of apoptosis induction as measured by Annexin V and PI staining and by PARP cleavage was consistent with the decrease in cell viability (Figure 1B-C). PARP, a substrate of caspase protease in an apoptosis-signaling pathway, was cleaved by 2 μM As_2O_3 treatment in Raji, but not in Jurkat, cells.

GSTP1 level, but not that of GSTA1 or GSTM1, is inversely correlated with As_2O_3-induced cell cytotoxicity

We used Western and Northern blot analyses to determine GSTP1-1, GSTA1-1, and GSTM1-1 protein levels and *GSTP1* mRNA levels in Jurkat and Raji cells. Jurkat cells expressed higher levels of GSTP1-1 protein and *GSTP1* mRNA than did Raji cells (Figure 2). GSTM1-1 protein expression was almost the same in both cell types and appeared not to contribute to the total GST activity using CDNB as a substrate.[22] GSTA1-1 protein was not detectable in either cell line (data not shown). GSH level was the same in both cell lines (data not shown). Jurkat cells had higher levels of GSTP1-1 activity than Raji cells (Figure 2C), suggesting that the basal GSTP1 level might determine cell sensitivity to As_2O_3-induced apoptosis.

Forced expression of *GSTP1* decreases cell sensitivity to As_2O_3-induced apoptosis

To investigate the role of *GSTP1* in the regulation of cell sensitivity to As_2O_3, Raji cells were stably transfected with a plasmid with or without a *GSTP1* expression sequence. Two Raji cell clones with *GSTP1* expression (RG19 and RG20) and 2 Raji clones without *GSTP1* expression (RV5 and RV7) were selected for further study. RG19 and RG20 cells had high GSTP1-1 activity—up to 120 nmol CDNB/min per milligram protein—which is similar to that in Jurkat cells, whereas RV5 and RV7 cells had low GSTP1-1 activity (less than 20 nmol CDNB/min per milligram protein) (Figure 3). Western blot analysis demonstrated that RG19 and RG20, but not

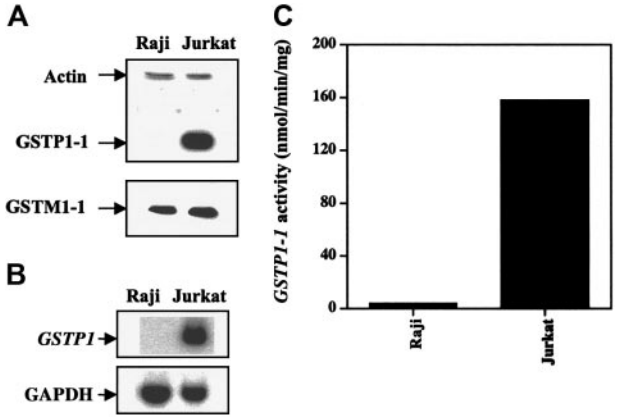

Figure 2. GSTP1 protein *GSTP1* and mRNA levels and activity in Raji and Jurkat cells. (A) Western blot analysis of GSTP1-1 and GSTM1-1 proteins. (B) Northern blot analysis of *GSTP1* mRNA. (C) *GSTP1-1* activity.

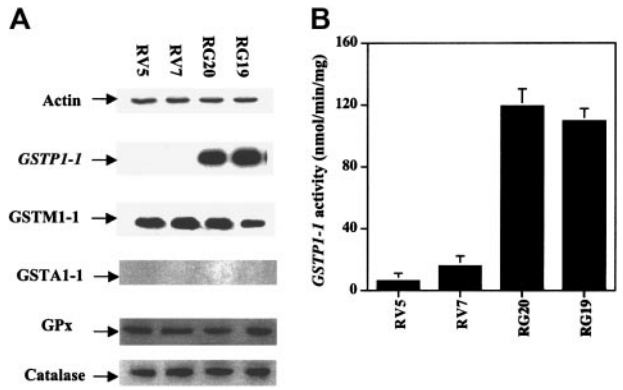

Figure 3. Generation of *GSTP1*-transfected Raji cell clones. Raji cells were transfected with a PcDNA3.1 plasmid with or without an inserted *GSTP1* cDNA sequence. Two clones expressing *GSTP1* (RG19 and RG20) and *2* without (RV5 and RV7) were selected by Western blot analysis (A) and activity assays (B), as described in "Materials and methods."

RV5 and RV7, expressed *GSTP1* mRNA and protein (Figure 3). Moreover, these 4 clones contained similar amounts of GSTM1-1, GSTA1-1, catalase, and glutathione peroxidase (GPx) based on Western blot analysis (Figure 3). As in parental Raji cells (Figure 1), As_2O_3 (2 μM) treatment induced apoptosis (23%) and reduced cell viability in RV7 cells (Figure 4). The apoptosis induction ability of As_2O_3 was partially blocked in *GSTP1*-transfected RG20 cells (Figure 4). These data suggest that *GSTP1* might be one of the factors inhibiting As_2O_3-induced apoptosis.

GSTP1 overexpression decreases H_2O_2 accumulation from As_2O_3 treatment in Raji cells

Amounts of intracellular H_2O_2 were determined in Jurkat, Raji, and *GSTP1*-expressing Raji cells. The basal H_2O_2 level in Jurkat cells was lower than it was in Raji cells. As_2O_3 (2 μM) treatment increased H_2O_2 levels in Raji cells, but not in Jurkat cells, after 3 days of treatment (Figure 5A). The effect of *GSTP1* on intracellular H_2O_2 levels in Raji clones grown in medium with or without 2 μM As_2O_3 for 48 and 72 hours was tested. RG20 and RG19 cells had lower intracellular H_2O_2 basal levels than RV5 and RV7 cells. H_2O_2 levels in RV5 and RV7 cells exposed to 2 μM As_2O_3 obviously increased at 48 hours and then continuously accumulated, up to as much as 5-fold, at 72 hours compared with untreated cells (Figure 5B), similar to the increases in parental Raji cells. However, H_2O_2 levels in RG20 and RG19 cells were only slightly increased at 48 hours and were 3-fold lower than those in RV5 and RV7 cells at 72 hours after the addition of 2 μM As_2O_3 (Figure 5B). Intracellular GSH levels were tested before and after As_2O_3 treatment in RV5 and RG19 cells. As_2O_3 treatment increased intracellular GSH content in RV5 cells, but not in RG19 cells (Figure 5C). After exogenous H_2O_2 was added to the growth medium, the elimination rate of H_2O_2 in RV5 and RG19 cells was determined. H_2O_2 was eliminated more rapidly from RG19 cells than from RV5 cells (Figure 5D). Mean levels of intracellular H_2O_2 2 hours after the addition of 500 μM H_2O_2 to the growth medium were 79.6 in RV5 cells but only 39.5 in RG19 cells. Under the same conditions, the levels of GSH were much lower after H_2O_2 treatment in RG19 cells (Figure 5E). These data suggest that As_2O_3 treatment results in the accumulation of H_2O_2 by inhibiting GSH-involved peroxidase activity, whereas GSTP1-1 may compromise an alternative GSH-involved peroxidase pathway not inhibited by As_2O_3 treatment.

GSTP1 overexpression decreases intracellular retention of ^{73}AsIII

To test the role of *GSTP1* on the cellular accumulation of As_2O_3, intracellular arsenic uptake was measured using ^{73}AsIII–arsenite. Similar ^{73}As uptake was found in *GSTP1*-expressing or -nonexpressing Raji cells after incubation for 1 hour (data not shown). Percentages of intracellular ^{73}As in RV5 and RV7 cells were 1.5- to approximately 2-fold higher than in RG20 and RG19 cells after incubation for 24 hours (Figure 6). These data indicate that overexpression of *GSTP1* decreases As retention in the cells.

GSTP1 overexpression decreases P-JNK after treatment with high but not therapeutic concentrations of As_2O_3

P-JNK is not detectable by Western blot analysis in any of the untreated cells. The phosphorylated form of JNK was not detected in either RV7 or RG20 cells after treatment with 1 to approximately 10 μM As_2O_3 for 72 hours, although apoptotic cells were detected (data not shown; Figure 4). However, high concentrations of As_2O_3 (approximately 50-80 μM) treatment for 2 hours activated the phosphorylation of JNK in RV7 cell clones and, to a lesser extent, in RG20 cells (Figure 7B). Moreover, phosphorylation of p38 was markedly increased after treatment with high concentrations of As_2O_3 (approximately 50-80 μM), and there was no detectable difference in p-p38 levels between *GSTP1*-expressing and -nonexpressing Raji cells (Figure 7). These data suggest that JNK activation represents a stress response and would not contribute to the apoptosis observed in Raji cells after treatment with As_2O_3 at therapeutic concentrations.

Discussion

Jurkat cells were less sensitive to As_2O_3-induced apoptosis than Raji cells, and Jurkat cells express higher levels of *GSTP1* mRNA and activity than Raji cells, which are devoid of *GSTP1* expression and activity (Figures 1, 2). Because other potential factors, such as GSTM1, catalase, GPx, and GSH levels, were equally expressed in both cell lines (Figure 3) and GSTA1 was absent, it appears that *GSTP1* might be the factor that mediates the observed different cell

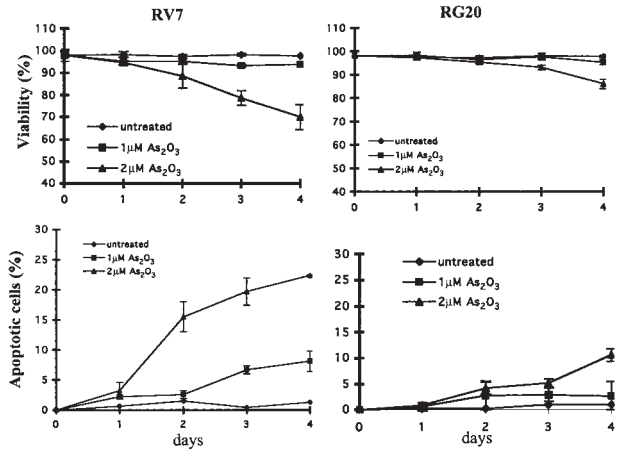

Figure 4. Cytotoxicity and apoptosis induction in *GSTP1*-transfected Raji clones. *GSTP1*-transfected RG20 cells and empty vector–transfected RV7 cells were untreated or treated with As_2O_3 at the indicated concentrations for indicated times. Cytotoxicity (A) was determined by trypan blue staining, and relative levels of apoptotic cells (B) were determined on flow cytometry by Annexin V and PI staining, as described in "Materials and methods."

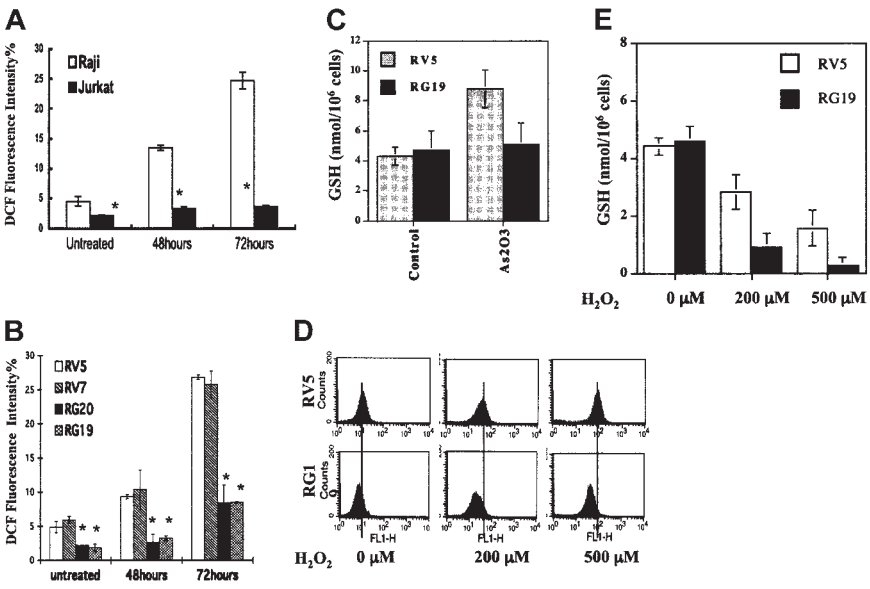

Figure 5. H_2O_2 and GSH levels in Raji and *GSTP1*-transfected Raji cells before and after As_2O_3 treatment. (A) Relative H_2O_2 levels in Jurkat and Raji cells. Growth medium was supplemented with 2 μM As_2O_3. After 48 and 72 hours, the intracellular level of H_2O_2 was measured by flow cytometry, as described in "Materials and methods." *Statistically significant ($P < .05$) differences, compared with Jurkat cells. (B) Relative H_2O_2 amount in *GSTP1*-transfected Raji clones. Growth medium was supplemented with 2 μM As_2O_3. After 48 and 72 hours, the intracellular levels of H_2O_2 were measured by flow cytometry. *Statistically significant ($P < .05$) differences, compared with RV5 and RV7 cell clones. (C) GSH levels in *GSTP1*-transfected Raji clones. Growth medium was supplemented with 2 μM As_2O_3. After 48 hours, the intracellular level of GSH was measured, as described in "Materials and methods." (D) H_2O_2 levels and (E) GSH levels after addition of exogenous H_2O_2 at the indicated concentrations into the growth medium of *GST*P1-expressing and -nonexpressing Raji cells. After 2 hours, intracellular H_2O_2 and GSH levels were measured, as described in "Materials and methods."

sensitivities to As_2O_3. Stable transfection with *GSTP1* in Raji cells decreased As_2O_3-induced apoptosis, suggesting that *GSTP1* is indeed a potent inhibitor of As_2O_3-induced apoptosis (Figure 4).

It has been found that As_2O_3-induced apoptosis at therapeutic concentrations is associated with the up-regulation of H_2O_2.[4-8] As_2O_3 treatment significantly increased H_2O_2 levels in parental Raji cells but not in Jurkat cells (Figure 5). Raji cells transfected with *GSTP1* had reduced levels of H_2O_2 production compared with vector-transfected cells. Moreover, given that vector-transfected cells contained relatively higher levels of H_2O_2 than *GSTP1*-transfected cells and that H_2O_2-scavenging enzymes, such as catalase, glutathione peroxidase, and GSTA1-1 were not changed in these cells (Figure 3), it appears that *GSTP1* may function as a peroxidase to diminish intracellular H_2O_2. *GSTA1,* but not *GSTP1,* has been reported to have selenium-independent glutathione peroxidase activity.[23-26] Studies have reported that *GSTP1* plays an important role in the detoxification of carcinogens and the prevention of DNA damage but not in H_2O_2 scavenging.[27-29] However, the observations that less H_2O_2 accumulation (Figure 5D) and more GSH depletion (Figure 5E) in Raji cells expressing *GSTP1* treated with H_2O_2 support the hypothesis that *GSTP1* can function as a glutathione-dependent peroxidase. The nature of *GSTP1* must be further studied by chemical methods.

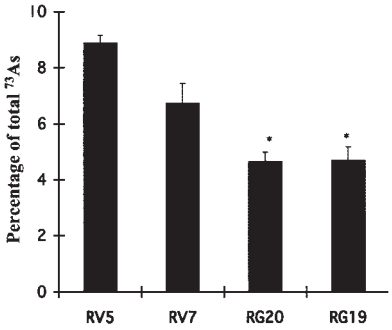

Figure 6. Expression of *GSTP1* decreases As_2O_3 retention. Raji cells expressing *GSTP1* (RG19 and RG20) and cells containing a vector not expressing *GSTP1* (RV5 and RV7) were incubated in medium with 1 μM ^{73}AsIII for 24 hours. Cell-associated radioactivity was determined as described in "Materials and methods." Each value represents the mean ± SD of triplicates. *Statistically significant ($P < .05$) differences in RG20 and RG19 cells, compared with those in RV5 and RV7 cell clones.

GSTP1 has also been reported to be involved in the detoxification of arsenic by an efflux system.[15,17,30] Increased *GSTP1* expression levels and activity were observed in arsenic-tolerant and -resistant cells.[14-18] It is possible that *GSTP1* facilitates the efflux of arsenite in the cells expressing *GSTP1;* in turn, GPx is not inhibited, and H_2O_2 does not accumulate after As_2O_3 treatment. The retention of As_2O_3 in Raji clones expressing *GSTP1* was less than it was in Raji clones not expressing *GSTP1* (Figure 6). That H_2O_2 did not significantly increase in *GSTP1*-expressing Raji cells after As_2O_3 treatment supports this possibility.

It has been reported that high concentrations of As_2O_3 treatment activated JNK and p38, members of stress-activated signal transduction pathways, and resulted in apoptosis in several leukemia and lymphoma cell lines.[11,12,31,32] Basal levels of the phosphorylated form of JNK and p38 were not detectable in Raji cell clones with or without *GSTP1* expression. Although a significant apoptotic effect was observed after treatment with 2 μM As_2O_3 in vector-transfected Raji cells, activation of JNK and p38 was still not detectable in these cells, suggesting that JNK and p38 activation might not contribute to As_2O_3-induced apoptosis at therapeutic concentrations. Furthermore, as reported by other groups,[10-13] the phosphorylated forms of JNK and p38 were significantly increased by As_2O_3 treatment at higher concentrations (Figure 7B), suggesting that JNK and p38 activation might mediate a stress response. Recently, it was found that *GSTP1* is an inhibitor of JNK activity through direct protein–protein interaction.[33] Forced expression of *GSTP1* in Raji cells decreased JNK phosphorylation after exposure to high concentrations of As_2O_3, which is consistent with previous reports that *GSTP1* inhibited JNK activation after stress treatments (high-dose H_2O_2 and As_2O_3). The phosphorylated form of p38 was equally increased after exposure to high concentrations of As_2O_3 treatment in Raji cells expressing or not expressing *GSTP1* (Figure 7). These data suggest that under stress condition after high As_2O_3 treatments, *GSTP1* may function as an inhibitor of JNK rather than of p38 kinase. However, at therapeutic As_2O_3 concentrations, caspase activation (Figure 1) but not JNK-mediated pathway(s) is correlated with apoptosis induction in lymphoma cells.

In conclusion, the data presented here indicate that *GSTP1* might be an important factor in determining the cell sensitivity

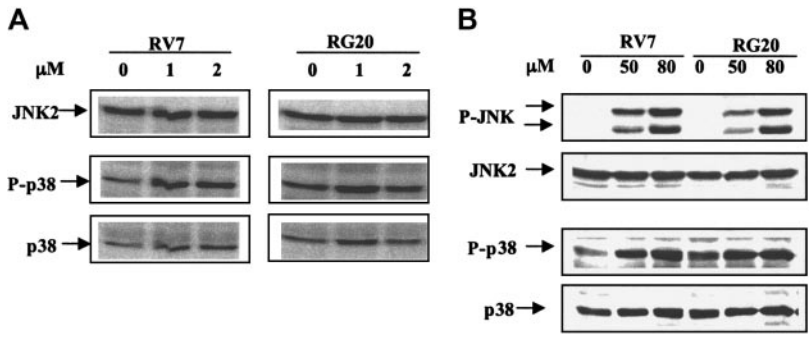

Figure 7. JNK was activated by higher, but not lower, therapeutic concentrations of As_2O_3, in transfected Raji cells with or without *GSTP1* expression. RV7 and RG20 cells were untreated or treated with 1 or 2 μM As_2O_3 for 24 hours (A) or 50 to 80 μM As_2O_3 for 2 hours (B). P-JNK, JNK, P-p38, and p38 were detected by Western blot analysis.

to As_2O_3-induced apoptosis in lymphoma. *GSTP1* may inhibit arsenic-induced apoptosis through at least 2 mechanisms: the detoxification mechanism, which decreases arsenic intracellular retention, and the peroxidase mechanism, which catabolizes H_2O_2. Each mechanism results in a decrease in intracellular H_2O_2 level and in the inhibition of apoptosis observed in Raji cells transfected with *GSTP1* and Jurkat cells after low-concentration As_2O_3 treatment.

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