Methylated Metabolites of Arsenic Trioxide Are More Potent Than Arsenic Trioxide as Apoptotic but not Differentiation Inducers in Leukemia and Lymphoma Cells

Guo-Qiang Chen, Li Zhou, Miroslav Styblo, Felecia Walton, Yongkui Jing, Rona Weinberg, Zhu Chen, and Samuel Waxman

Department of Pathophysiology [G-Q.C.] and Shanghai Institute of Hematology [Z. C.]; Shanghai Second Medical University, Shanghai, China; Division of Hematology/Oncology, Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029 [L. Z., Y. J., R. W., S. W.]; and Department of Pediatrics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina [M. S., F. W.]

ABSTRACT

Treatment with arsenic trioxide (As2O3) by inducing apoptosis and partial differentiation of acute promyelocytic leukemia (APL) cells results in clinical remission in APL patients resistant to chemotherapy and all-trans-retinoic acid. As2O3 (iAsIII) is methylated in the liver to mono- and dimethylated metabolites, including methylarsonic acid, methylarsonous acid, dimethylarsinic acid, and dimethylarsinous acid. Methylated trivalent metabolites that are potent cytotoxins, genotoxins, and enzyme inhibitors may contribute to the in vivo therapeutic effect of iAsIII. Therefore, we compared the potency of iAsIII and trivalent metabolites using chemical precursors of methylarsonous acid and dimethylarsinous acid to induce differentiation, growth inhibition, and apoptosis. Methylarsine oxide (MAsIII O) and to a lesser extent iododimethylarsine were more potent growth inhibitors and apoptotic inducers than iAsIII in NB4 cells, an APL cell line. This was also observed in K562 human leukemia, lymphoma cell lines, and in primary culture of chronic lymphocytic leukemia cells, but not human bone marrow progenitor cells. Apoptosis was associated with greater hydrogen peroxide accumulation and inhibition of glutathione peroxidase activity. MAsIII O, in contrast to iAsIII, did not induce PML-retinoic acid receptor α degradation, or restore PML nuclear bodies or differentiation in NB4 cells. In a cocultivation experiment, hepatoma-derived HepG2 cells, but not NB4 cells, methylate radiolabeled iAsIII. Methylated metabolites released from HepG2 cells are preferentially accumulated by NB4 cells. This experimental model suggests that in vivo hepatic methylation of iAsIII may contribute to As2O3-induced apoptosis but not differentiation of APL cells. MAsIII O as an apoptotic inducer should be considered in the treatment of other hematologic malignancies like lymphoma.

INTRODUCTION

APL is an unique subtype of acute myeloid leukemia typically carrying the specific reciprocal chromosomal translocation t(15;17) leading to the expression of the leukemia-generating fusion protein PML-RARα (1, 2). In 90% of de novo APL patients, ATRA treatment induces differentiation of leukemic blasts and clinical remission, and 70% have been cured by ATRA in combination with chemotherapy (3). There is a 30% relapse, especially in APL patients presenting with high WBC. However, treatment with As2O3 induces complete remission in 90% of this group in combination of chemotherapy and ATRA-resistant relapsed patients (4–6). This suggests that As2O3 treatment may induce remission in APL patients by a mechanism different from ATRA or chemotherapy. Indeed, only differentiated leukemia cells have been observed in APL patients after ATRA treatment (7), but both apoptotic and partially differentiated cells have been observed in APL patients and animal models treated with As2O3 (5, 8). This suggests that As2O3 may induce clinical remission in APL patients by multiple mechanisms.

Biomethylation is the major metabolic pathway for iAs in humans and many animal species (9). In this pathway iAs undergo metabolic conversion that includes reduction of iAsV to iAsIII with subsequent methylation yielding mono- and dimethylated metabolites (10, 11). The postulated scheme is as follows: iAsV → iAsIII → MAsV → MAsIII O → DMAsV → DMAsIII O → MAsIII and DMAsIII have been detected in the urine of humans exposed to inorganic arsenic in drinking water (11, 12) and in human hepatoma (HepG2) cells exposed to iAsIII (11). Notably, chemical derivatives of MAsIII are significantly more toxic for cultured cells (13, 14) and laboratory animals (15) than either iAsV or iAsIII. DMAsIII derivatives are more toxic than iAs species in most cell types tested (13, 14). Although As2O3 has been found to induce remission in APL patients and induce apoptosis in cultured APL cells (4, 5, 8, 16), the metabolism of As2O3 and the effect of its methylated metabolites have not been tested. It is possible that methylated As2O3 metabolites that form in vivo may contribute to the therapeutic effect of As2O3 in APL.

NB4 cells, a cell line derived from t(15;17) APL (17–20) were used to compare the induction of apoptosis and differentiation by iAsIII to methylated arsenicals that are chemical precursors of methylated trivalent metabolites of iAs, MAsIII O and DMAsIII I. The effects of these arsenicals on H2O2 production, glutathione peroxidase activity, caspase activation, PML-RARα protein degradation, and PML nuclear body formation were also examined. We also studied the production and distribution of iAsIII metabolites in a cocultivation cell culture system containing HepG2, a hepatoma-derived cell line and NB4 cells. The cytotoxic effect of MAsIII O and iAsIII was also compared in K562 leukemia cells, lymphoma cells, primary cultures of CLL cells, and in normal human bone marrow progenitor cells. The data indicate that MAsIII O and to a lesser extent DMAsIII I, were more potent growth inhibitors and apoptotic inducers than iAsIII in leukemia and lymphoma cells but not in human bone marrow progenitor cells. H2O2 accumulation and GPx inhibition but not degradation of PML-RARα correlated with greater MAsIII O-induced apoptosis in NB4 cells. NB4 cells did not methylate iAsIII, but mono- and dimethylated metabolites formed from iAsIII in HepG2 cells released into the medium and were preferentially taken up by NB4 cells.
MATERIALS AND METHODS

Arsenicals and Reagents. iAsIV and iAsV, sodium salts (at least 99% pure), were purchased from Sigma (St. Louis, MO). iAsIII (sodium salt, 98% pure) was obtained from Chem Service (West Chester, PA) and DMAVIII (98% pure) from Strem (Newburyport, MA). MAsVIII (CH3AsO) and DMAVII[(CH3)2AsI] were synthesized by Dr. William R. Cullen (University of British Columbia, Vancouver, British Columbia, Canada) using methods described previously (21, 22). Identity and purity of these compounds were confirmed using 1H NMR and mass spectrometry. In aqueous solutions, MAsVII and DMAVII form the respective (methylarsonous and dimethylarsenous) oxyanions. The hydrolysis of oxides and iodides of MASIII has been documented previously by Petrick et al. (15) using 1H NMR and mass spectrometry. Carrier-free [73As]iAsII was purchased from Los Alamos Meson Products Division, Los Alamos, NM. [73As]iAsVII was prepared from [73As]iAsIII by reduction with metabisulfite/thiosulfate reagent (23). Yields of [73As]iAsIII in this reaction as determined by TLC (24) typically exceed 95%.

Cell Culture and Treatment. NB4 human APL cells (kindly provided by Dr. M. Lanotte, Hopital Saint-Louis, Paris, France; Ref. 17) and the NB4-derived ATTRA-resistant R4 line (kindly provided by W. Miller, Jr., Lady Davis Institute for Medical Research, Montreal, Canada; Ref. 25) and K562 cells (obtained from ATCC) were cultured in RPMI 1640 supplemented with 10% FCS (JRHI BioScience, Lenexa, KS) in a humidified atmosphere of 95% air and 5% CO2 at 37°C. HepG2 cells (obtained from ATCC) were cultured in MEM supplemented with 10% FCS. Jurkat and Namalwa human lymphoma cells (obtained from ATCC) were cultured in RPMI 1640 adjusted to contain 1.5 g/liter of sodium bicarbonate, 4.5 g/liter of glucose, 10 mM HEPES, 1.0 mM sodium pyruvate and 10% FCS. CLL cells were obtained from blood from two patients with consent according to Institutional Review Board regulations. B-cell populations were isolated using RosetteSep B Cell Enrichment mixture (StemCell Technologies, Vancouver, British Columbia, Canada), which used antibodies against CD2, CD3, CD16, CD36, and CD56. This negative selection procedure cannot determine original oxidation states of arsenic in metabolites. Thus, arsenic metabolites in this series of experiments are generically referred to as iAs, MAS, and DMAs.

Biochemical Assays. Cell-Free Assays. Glutathione peroxidase (GSH-Px) activity was determined using commercial kits (Calbiochem, San Diego, CA). One milliunit of enzyme activity was defined as 1 nmol of NADH oxidized to NADP per milligram of protein per min.

RESULTS

Methylation of iAsIII Increases Cytotoxicity in NB4 Cells. We compared growth inhibition and cell death by treatment with iAsIII, iAsV, and their monomethylated and dimethylated derivatives in NB4 cells at concentrations of 0.5, 1, and 2 μM for 1–3 days. MAsVIII treatment was more growth inhibitory (IC50 = 0.3 μM) than iAsIII, whereas DMAsVII was similar to iAsIII (IC50 ~1.0 μM) at 3-day treatment (Fig. 1A). There was minimal loss of viability after treatment with 0.5 μM of iAsIII, MAsVIII, and iAsVIII. However, 1 μM of MAsVIII caused 90% loss of viability of NB4 cells, whereas iAsIII and DMAsVII remained at 15% (Fig. 1B). Treatment with up to 2 μM of pentavalent methylated arsenicals (MAS and DMAS) did not significantly inhibit growth or decrease viability of NB4 cells (data not shown).
MAs\textsubscript{III}O and DMA\textsubscript{III}I Are More Potent Apoptotic Inducers Than iAs\textsubscript{III} in NB4 Cells. Induction of apoptosis as measured by Annexin-V/PI assay was greatest after MAs\textsubscript{III}O treatment of NB4 cells. One μM of iAs\textsubscript{III}, MAs\textsubscript{III}O, and DMA\textsubscript{III}I induced apoptotic and necrotic cells to 10, 63, and 21% in NB4 cells after 2 days treatment, respectively (Fig. 2A). Similarly, K562 human leukemia cells are more growth inhibited (IC\textsubscript{50} 0.7 μM versus 2.1 μM) and undergo greater apoptosis (12.5 versus 1.8%) by 48 h of treatment with 1 μM of MAs\textsubscript{III}O as compared with iAs\textsubscript{III}. The extent of apoptosis induced by MAs\textsubscript{III}O and DMA\textsubscript{III}I correlated with more effective PARP cleavage (Fig. 2B).

Production of H\textsubscript{2}O\textsubscript{2} and Inhibition of GPx Activity by MAs\textsubscript{III}O Correlates with Higher Apoptotic Activity. We reported previously that iAs\textsubscript{III}-induced apoptosis followed accumulation of H\textsubscript{2}O\textsubscript{2} and inhibition of GPx activity. Therefore, it is likely that MAs\textsubscript{III}O, which is more potent than iAs\textsubscript{III} in inducing apoptosis, also produces more H\textsubscript{2}O\textsubscript{2} and inhibits GPx activity, resulting in a higher degree of apoptosis.

![Fig. 1. Effects of trivalent arsenicals on the growth of NB4 cells. A. NB4 cells were treated with 0.5–2.0 μM iAs\textsubscript{III} (top), MAs\textsubscript{III}O (middle), and DMA\textsubscript{III}I (bottom) for the indicated days. Total cell number was counted. Each point is the mean of triplicates with ±5% variation (data not shown). B. Effects of trivalent arsenicals on viability of NB4 cells. Viable cell numbers were counted by trypan-blue exclusion. All data represent an independent experiment from two repeated tests with similar results. Each point is the mean of triplicates, bars, ±SD.](image1)

![Fig. 2. Apoptosis induced by arsenicals in NB4 cells. A. NB4 cells were treated with 1 μM iAs\textsubscript{III}, MAs\textsubscript{III}O, and DMA\textsubscript{III}I, respectively, for 24 and 48 h. Annexin-V and PI staining were measured by flow cytometry. B. Western blot analysis of PARP cleavage. Antibody to PARP was used to detect the PARP cleavage product.](image2)
Thus, these data demonstrate that degradation of PML-RAR\(\text{H9251}\) in NB4 cells, and partially reformed PML nuclear bodies (Fig. 4) microscopy using PML antibody demonstrated that iAs\(\text{III}\) treatment\(\text{H11011}\) 10-fold more H\(_2\)O\(_2\) accumulation than iAs\(\text{III}\) (Table 1) and corelated with the extent of GPx inhibition. These data support the idea that MAs\(\text{III}\), like As\(\text{III}\), induces apoptosis in APL cells by a H\(_2\)O\(_2\)-mediated pathway.

Some lymphoma cells are also sensitive to low concentration of As\(_2\)O\(_3\)-induced apoptosis. As shown in Fig. 5A, MAs\(\text{III}\) is a more potent cytotoxic agent in human lymphoma cells. Some lymphoma cells are also sensitive to low concentration of As\(_2\)O\(_3\)-induced apoptosis (30). The ability of MAs\(\text{III}\) to induce growth inhibition and cell death was studied in two lymphoma cell lines: Jurkat, which are insensitive, and Namalwa cells, which are sensitive to As\(_2\)O\(_3\)-induced apoptosis. As shown in Fig. 5A, MAs\(\text{III}\) was more growth inhibitory than iAs\(\text{III}\) in both Jurkat and Namalwa cells, because treatment with 1 \(\mu\)M iAs\(\text{III}\) resulted in <10\%, whereas MAs\(\text{III}\) resulted in >50\% growth inhibition of both lymphoma cell lines after 3 days of treatment. This was reflected by a greater loss of cell viability after MAs\(\text{III}\) treatment (Fig. 5B).

CLL cells in primary cultures are minimally affected by treatment with 2 \(\mu\)M iAs\(\text{III}\) for 3 days. In contrast, 0.5 \(\mu\)M MAs\(\text{III}\)O treatment for 1 day inhibits growth (48\%) and decreases viability (25\%), and after 3 days there is 85\% loss of viability (Table 2).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Relative H(_2)O(_2) amount in NB4 cells after arsenical treatment(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 H</td>
</tr>
<tr>
<td>Control</td>
<td>0.71 ± 0.15</td>
</tr>
<tr>
<td>1 (\mu)M iAs(\text{III})</td>
<td>4.24 ± 1.33</td>
</tr>
<tr>
<td>1 (\mu)M MAs(\text{III})O</td>
<td>37.57 ± 1.15</td>
</tr>
<tr>
<td>1 (\mu)M DMA(\text{III})</td>
<td>24.97 ± 0.45</td>
</tr>
</tbody>
</table>

\(^a\)H\(_2\)O\(_2\) amount was measured by FACS.

inhibition of GPx activity (20). GPx activity was partially inhibited by iAs\(\text{III}\) treatment of NB4 cells in a time- and concentration-dependent manner. However, GPx activity was more rapidly inhibited and to a much greater extent by 0.5 \(\mu\)M MAs\(\text{III}\)O than by 2 \(\mu\)M iAs\(\text{III}\) in NB4 cells (Fig. 3). MAs\(\text{III}\)O (1 \(\mu\)M) treatment for 24 or 48 h caused ~10-fold more H\(_2\)O\(_2\) accumulation than iAs\(\text{III}\) (Table 1) and correlates with the extent of GPx inhibition. These data support the idea that MAs\(\text{III}\)O, like As\(\text{III}\), induces apoptosis in APL cells by a H\(_2\)O\(_2\)-mediated pathway.

MAs\(\text{III}\)O-induced Apoptosis Is Independent of PML-RAR\(\alpha\) Protein Degradation. To evaluate the role of PML-RAR\(\alpha\) protein degradation in MAs\(\text{III}\)O-induced apoptosis, we measured PML-RAR\(\alpha\) levels by Western blot analysis and PML levels by immunocytochemistry using confocal microscopy. NB4 cells treated with 1 \(\mu\)M MAs\(\text{III}\)O for 24 h produced more apoptosis than treatment with 1 \(\mu\)M iAs\(\text{III}\) (Fig. 2A). However, iAs\(\text{III}\) but not MAs\(\text{III}\)O treatment degraded PML-RAR\(\alpha\) in NB4 cells after 24 h (Fig. 4A). Furthermore, confocal microscopy using PML antibody demonstrated that iAs\(\text{III}\) treatment but not MAs\(\text{III}\)O reversed the abnormal microspeckled pattern found in NB4 cells, and partially reformed PML nuclear bodies (Fig. 4B). Thus, these data demonstrate that degradation of PML-RAR\(\alpha\) protein is not a requirement for the more effective MAs\(\text{III}\)O-induced apoptosis.

PML-RAR\(\alpha\) protein degradation after iAs\(\text{III}\) treatment in vitro is thought to be a requirement for differentiation induction of ATRA-sensitive and -resistant APL cells (29). As shown in Fig. 4C, iAs\(\text{III}\)- but not MAs\(\text{III}\)O-treated NB4 cells undergo partial differentiation as measured by CD11b using FACS analysis.

MAs\(\text{III}\)O Is a More Potent Cytotoxic Agent in Human Lymphoma Cells. Some lymphoma cells are also sensitive to low concentration of As\(_2\)O\(_3\)-induced apoptosis (30). The ability of MAs\(\text{III}\) to induce growth inhibition and cell death was studied in two lymphoma cell lines: Jurkat, which are insensitive, and Namalwa cells, which are sensitive to As\(_2\)O\(_3\)-induced apoptosis. As shown in Fig. 5A, MAs\(\text{III}\) was more growth inhibitory than iAs\(\text{III}\) in both Jurkat and Namalwa cells, because treatment with 1 \(\mu\)M iAs\(\text{III}\) resulted in <10\%, whereas MAs\(\text{III}\) resulted in >50\% growth inhibition of both lymphoma cell lines after 3 days of treatment. This was reflected by a greater loss of cell viability after MAs\(\text{III}\) treatment (Fig. 5B).

CLL cells in primary cultures are minimally affected by treatment with 2 \(\mu\)M iAs\(\text{III}\) for 3 days. In contrast, 0.5 \(\mu\)M MAs\(\text{III}\)O treatment for 1 day inhibits growth (48\%) and decreases viability (25\%), and after 3 days there is 85\% loss of viability (Table 2).
controls. The combined and individual cultures were exposed to 0.1 or 1 μM arsenite (iAs III) and DMAs III O. Individual cultures of HepG2 and NB4 cells were used as cocultivation experiments in combined cultures of HepG2 and NB4 cells. To examine the affinity of APL cells toward natural metabolites of iAs, we carried out cocultivation experiments in a cocultivation cell culture system.

Effect of iAs III and MAs III O on Normal Hematopoietic Cells. Human bone marrow MNCs (n = 3) grown in methylcellulose were treated with iAs III or MAs III O, as reported previously (31). CFU-E-, BFU-E-, and CFU-GM-derived colony growth was unaffected by doses of 0.1-1 μM iAs III. A similar level of toxicity was observed with up to 1 μM MAs III O treatment (Fig. 6). However, erythroid colonies (CFU-E and BFU-E) but not granulocytic colonies (CFU-GM) were more inhibited by 2 μM MAs III O than 2 μM iAs III.

Metabolism of iAs III and Distribution of Methylated Metabolites in a Cocultivation Cell Culture System. To examine the affinity of APL cells toward natural metabolites of iAs, we carried out cocultivation experiments in combined cultures of HepG2 and NB4 cells. Individual cultures of HepG2 and NB4 cells were used as controls. The combined and individual cultures were exposed to 0.1 or 1 μM [73 As]iAs III for 48 h (Table 3; Ref. 4). Radiolabeled metabolites of [73 As]iAs III were analyzed in culture medium, HepG2 cells, and/or NB4 cells separately. No methylated metabolites were detected in the individual NB4 cultures regardless of the iAs III concentration. U937 human leukemia cells like NB4 cells take up but do not methylate [73 As]iAs III (data not shown). In the individual and combined cultures containing HepG2 cells, [73 As]iAs III was methylated to yield radiolabeled MAs and DMAs. DMAs was the major methylated metabolite in HepG2 cultures exposed to 0.1 μM arsenite (Table 3). On the other hand, MAs was the predominant methylated metabolite in cultures exposed to 1 μM arsenite (Table 4). In the combined cultures, significant portions of MAs and DMAs synthesized by HepG2 cells and released into the medium were found in NB4 cells. MAs retained in NB4 cells represented 73% of this metabolite released in culture medium by HepG2 cells when exposed to 1 μM iAs III. In contrast, only 8.5% of iAs in the medium was taken up by NB4 cells. In the combined cultures exposed to 1 μM iAs III, the ratio of iAs:MAs was 10-fold greater in the culture medium (36.8) than in NB4 cells (3.5). DMAs represented only a minor fraction of the total arsenic in NB4 cells regardless of treatment.

DISCUSSION

We established that NB4 cells can take up and accumulate methylated metabolites of iAs produced and released by HepG2 cells using a combined cell culture system (Tables 3 and 4). The methylation patterns of iAs in HepG2 cells resemble those reported in primary human hepatocytes (11, 27). Similar to primary cultures of hepatocytes, moderate (micromolar) concentrations of iAs III inhibit methylation reactions, particularly DMA synthesis, in HepG2 cells yielding the intermediary metabolite, MA, as the major methylated product (Tables 3 and 4). The lack of methylated metabolites in NB4 cultures exposed to either 0.1 or 1 μM iAs III suggests that these cells do not methylate iAs. In the combined cultures NB4 cells took up and accumulated significant portions of methylated metabolites, especially MAs, produced by HepG2 monolayers. The comparison of the ratios of metabolites in the culture medium and of metabolites retained in NB4 cells suggests that NB4 cells have a very high affinity for MAs as compared with other arsenic metabolites. The arsenic speciation techniques used in this study could not determine oxidation states (valencies) of arsenic metabolites. However, because pentavalent arsenicals are not effectively taken up by cultured cells (11, 27), MAs and DMAs retained in NB4 cells are most likely in the trivalent forms of MAs III and DMAs III.

Among all of the biologically relevant chemical forms of arsenic, a combined cell culture system (Tables 3 and 4). The methylation patterns of iAs in HepG2 cells resemble those reported in primary human hepatocytes (11, 27). Similar to primary cultures of hepatocytes, moderate (micromolar) concentrations of iAs III inhibit methylation reactions, particularly DMA synthesis, in HepG2 cells yielding the intermediary metabolite, MA, as the major methylated product (Tables 3 and 4). The lack of methylated metabolites in NB4 cultures exposed to either 0.1 or 1 μM iAs III suggests that these cells do not methylate iAs. In the combined cultures NB4 cells took up and accumulated significant portions of methylated metabolites, especially MAs, produced by HepG2 monolayers. The comparison of the ratios of metabolites in the culture medium and of metabolites retained in NB4 cells suggests that NB4 cells have a very high affinity for MAs as compared with other arsenic metabolites. The arsenic speciation techniques used in this study could not determine oxidation states (valencies) of arsenic metabolites. However, because pentavalent arsenicals are not effectively taken up by cultured cells (11, 27), MAs and DMAs retained in NB4 cells are most likely in the trivalent forms of MAs III and DMAs III.

Among all of the biologically relevant chemical forms of arsenic,
MAsIII derivatives are the most potent enzyme inhibitor, as shown here for GPx (Fig. 3), and cytotoxicity (reviewed in Ref. 32). In addition, MAsIII and DMAIII derivatives are considerably more effective than arsenite in nicking naked DNA in an in vitro system or damaging DNA in intact cells (33). These methylated trivalent arsenicals are also potent inducers of c-Jun phosphorylation and activator protein DNA binding in human cells (14, 34). The results of this study suggest that leukemia cells in vivo may accumulate MAsIII produced and released from the liver of patients treated with As₂O₃. These observations suggest that methylated metabolites of As₂O₃ may contribute to therapeutic and toxic effects in APL patients. To test this possibility, we compared the extent of growth inhibition and apoptosis induced in NB4 cells and K562 cells by treatment with iAsIII, and MAsIII and DMAIII chemical precursors of methylated trivalent metabolites of iAsIII (Figs. 1 and 2). Our data indicate that the apoptosis-induction ability is MAIII > DMAIII > iAsIII. MAIII treatment also inhibits growth and induces apoptosis to a greater extent than iAsIII. This suggests that the amount of MAIII in cells may mediate the sensitivity to As₂O₃-induced apoptosis in malignant cells and normal tissues such as bone marrow and liver. The explained and unusual severe liver toxicity in three Chinese patients treated for APL with As₂O₃ may relate to aberrant hepatocyte methylation of As₂O₃ (8). Studies to characterize hepatic arsenic methyltransferase polymorphism, isoforms, and nutritional cofactors are required to better understand As₂O₃ as a therapeutic agent.

We compared the uptake of ⁷³As arsenate and methylated arsenicals metabolites in NB4, which are sensitive to As₂O₃-induced apoptosis, and U937 cells, which are insensitive to As₂O₃-induced apoptosis (20). NB4 and U937 cells have similar accumulation of ⁷³As, do not methylate iAsIII, and take up MAsIII and DMAIII to similar amounts in cocultivation with HepG2 cells (Tables 3 and 4; data not shown). Thus, other intracellular factors contribute to the higher sensitivity of NB4 cells to MAIII-induced apoptosis. The intracellular content of glutathione, and GPx, glutathione S-transferase π, and catalase activities also play an important role in catabolism of H₂O₂. In NB4, cells with low levels As₂O₃ treatment induces H₂O₂ accumulation and apoptosis, and when high, there is less apoptosis in leukemic cells such as U937 cells (20). MAIII is a more potent H₂O₂ inducer than iAsIII (Table 1) and is known to have a stronger binding affinity to thiol-enzymes (21). GPx, which has a thiol-like group required for its activation (35), is more inhibited by MAIII (Fig. 3), which may contribute to the greater H₂O₂ accumulation (Table 1) and apoptosis.

It has been suggested that degradation of PML-RARα and PML protein is required for As₂O₃-induced apoptosis in APL cells (18, 19). This would predict a greater ability of MAIII than iAsIII to degrade PML-RARα or PML. However, PML-RARα protein degradation follows iAsIII but not MAIII treatment in NB4 cells (Fig. 4A). Moreover, iAsIII decreased abnormal PML nuclear body distribution and number as reported before (Fig. 4B), whereas neither MAIII nor DMAIII (data not shown) change the abnormal distribution of nuclear bodies in NB4 cells. Therefore, As₂O₃ triggers apoptosis by a pathway independent of PML or PML-RARα degradation, thus not limiting this therapeutic effect to APL.

The clinical response of APL patients to As₂O₃ treatment that is associated with differentiation induction is probably related to iAsIII (8). Unlike iAsIII, MAIII and DMAIII treatment of NB4 cells does not induce differentiation in NB4 cells (Fig. 4C) and does not cooperate with ATRA to induce differentiation in R4 ATRA-resistant NB4 cells (Ref. 25; data not shown). Therefore, the in vivo therapeutic contribution of MAIII would be the induction of apoptosis, not differentiation. The contribution of hepatic derived MAsIII from iAsIII to therapeutic outcome remains to be determined. MAIII released from hepatocytes after metabolism of iAsIII is avidly taken up by APL cells (Table 3; Ref. 4) and may contribute more than iAsIII to the induction of apoptosis, particularly in APL cells, which have a biochemical phenotype that renders them sensitive to H₂O₂-mediated apoptosis (18).

The finding of significant growth inhibition and apoptosis by treatment with As₂O₃ (16, 18–20), and to a greater extent by MAIII in lymphoma cells (Fig. 5) and in CLL cells in primary culture (Table 2) supports the evaluation of As₂O₃ and MAIIIO in additional clinical trials. MAIIIO, up to a concentration of 1 μM, although more potent than iAsIII to induce apoptosis in NB4 and death in lymphoma cells, does not show greater toxicity than iAsIII in human bone marrow as measured by colony assays (Fig. 6). We reported previously that the combination of ascorbic acid and As₂O₃, which increases H₂O₂ accumulation and apoptosis in NB4 and lymphoma cell lines, does not enhance As₂O₃ toxicity in human bone marrow as measured by colony assays (31). This correlates with a greater therapeutic effect of As₂O₃ in combination with ascorbic acid without increasing toxicity in a mouse lymphoma model (31). This may be related to the observation that human bone marrow progenitor cells have high catalase levels (36), which may render these cells more resistant to H₂O₂ accumulation after As₂O₃ treatment. The uptake and conversion of iAsIII to MAIIIO by HepG2 cells support the use of iAsIII or MAIIIO in the treatment of patients with hepatoma. There may be an increased potential for toxicity, particularly in liver with MAIIIO treatment. Thus, animal studies are required to evaluate this derivative before future clinical trials.

**ACKNOWLEDGMENTS**

We thank Dr. George Acs for critical reading of this manuscript.

**REFERENCES**


Methylated Metabolites of Arsenic Trioxide Are More Potent Than Arsenic Trioxide as Apoptotic but not Differentiation Inducers in Leukemia and Lymphoma Cells

Guo-Qiang Chen, Li Zhou, Miroslav Styblo, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/63/8/1853

Cited articles
This article cites 35 articles, 15 of which you can access for free at:
http://cancerres.aacrjournals.org/content/63/8/1853.full.html#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
/content/63/8/1853.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.