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

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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 monomethylated and dimethylated metabolites, including methylarsonic acid, methylarsonious acid, dimethylarsinic acid, and dimethylarsinous acid. Methylated trivalent metabolites 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 methylated metabolites using chemical precursors of methylarsonious 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, methylated radio-labeled iAsIII. Methylated metabolites released from HepG2 cells are preferentially accumulated by NB4 cells. This experimental model suggests that iAsIII in 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 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 iAsIII 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 → DMAsV → DMAsIII → MAsIII and DMAsIII have been detected in the urine of humans exposed to inorganic arsenic in drinking water (11) 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. DMAIII 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. 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, were more potent growth inhibitors and apoptotic inducers than iAsIII in leukemia and lymphoma cells but not in normal 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. iAs\textsuperscript{III} and iAs\textsuperscript{V}, sodium salts (at least 99% pure), were purchased from Sigma (St. Louis, MO), MAS\textsuperscript{III}O (CH\textsubscript{3}AsO) and DMAS\textsuperscript{III}I[(CH\textsubscript{3})\textsubscript{2}AsI] 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 \textsuperscript{1}H NMR and mass spectrometry. In aqueous solutions, the hydrolysis of oxides and iodides of MASIII has been documented previously (21, 22). Identity and purity of these compounds were confirmed using \textsuperscript{1}H NMR and mass spectrometry. In aqueous solutions, the hydrolysis of oxides and iodides of MASIII has been documented previously (21, 22).

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 generally referred to as iAs, MAS, and DMAs.

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 (JRH BioScience, Lenexa, KS) in a humidified atmosphere of 95% air and 5% CO\textsubscript{2} 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 system enabled the isolation of B cells that were of >95% purity by FACS analysis without activating the cells through any surface proteins. Whole blood was incubated with RosetteSep (50 μl of RosetteSep mixture/ml whole blood) at room temperature for 20 minutes. Samples were then diluted 1:2 with PBS +2% FCS and centrifuged over Ficoll-Hypaque density medium (Amersham Biosciences, Piscataway, NJ). B cells were collected from the Ficoll:plasma interface, washed in PBS, and ready for use. For treatment with arsenicals, cells were seeded at 2 × 10\textsuperscript{5} cells/ml and cultured in the above-mentioned medium with or without the indicated doses of compounds for the indicated time. Cell viability was estimated by trypan blue dye exclusion, and cell numbers were counted by hemocytometer.

Annexin-V Assay. Apoptotic cells were detected by Annexin-V assay. Generally 5–10 × 10\textsuperscript{5} cells after treatments were washed twice with PBS. Then, cells were labeled by Annexin-V-FITC and PI in binding buffer according to the instruction in the Annexin-V-FITC Apoptosis Detection kit provided by the manufacturer (Oncogene, Cambridge, MA). Fluorescent signals of FITC and PI were detected, respectively, by FL1 (FITC detector) at 518 nm and FL2 at 620 nm on FACSscan (Becton Dickson, San Jose, CA). The log of Annexin-V-FITC fluorescence was displayed on the X axis and the log of PI fluorescence on the Y axis, and data were analyzed using the CELLQuest (Becton Dickson) program. For each analysis, 10,000 events were recorded.

Measure of Intracellular Hydrogen Peroxide Production. The intracellular hydrogen peroxide level was detected as we reported before using 5,6-carboxy-2',7'-dichlorofluorescein-diacetate (Molecular Probe, Eugene, OR; Ref. 20). Briefly, 2 h before ending the indicated treatment, 10 μM 5,6-carboxy-2',7'-dichlorofluorescein-diacetate was added into the medium and continued incubation for 2 h at 37°C, and the fluorescence intensity was measured by FACSscan (Becton Dickson).

Glutathione Peroxidase Activity Assays. Cells (5 × 10\textsuperscript{3}) were washed twice with PBS, resuspended in PBS, sonicated for 10 s, centrifuged at 14,000 rpm for 10 min, and the supernatants subjected to enzyme assays. Glutathione peroxidase activity was determined using commercial kits (Calbiochem, San Diego, CA). One milliliter of enzyme activity was defined as 1 nmol of NADPH oxidized to NADP per milligram of protein per min.

Immunofluorescence and Confocal Laser Microscopy Analysis. Cells were smeared onto slides and kept on −80°C. For immunofluorescence staining of PML, which was described previously (26), cells were fixed in 20% methanol and antihuman PML monoclonal antibody 5E10 and then with FITC-conjugated secondary antibodies. Fluorescence was observed using a Leica fluorescence microscope with excitation at 488 nm (green). Images were overlaid in PhotoShop.

Metabolic Studies in a Cocultivation Culture System. HepG2 cells were cultured in monolayers in 12-well culture plates with MEM-supplemented 10% serum, and cells were cultured at 37°C in a humidified incubator in an atmosphere of 95% air and 5% CO\textsubscript{2} to 90% confluence (~1.6 × 10\textsuperscript{5} cells/well). Before exposure to iAs\textsuperscript{III}, MEM was replaced with a supplemented Williams’ medium E (27) with 10% fetal bovine serum (1.5 ml/well), and cultures were left to equilibrate for 1 h. The supplemented Williams’ medium E has been shown previously to provide an optimal support for methylation of arsenate in hepatic cells (27). Carrier-free \textsuperscript{73}As\textsuperscript{III}As\textsuperscript{III} was prepared from \textsuperscript{73}As\textsuperscript{III}As\textsuperscript{III} by reduction with metabisulfite/thiosulfate reagent (23). Yields of \textsuperscript{73}As\textsuperscript{III}As\textsuperscript{III} in this reaction as determined by TLC (24) typically exceed 95%.

Cellular hydrogen peroxide level was detected as we reported before by using acidic CuCl solution (pH 1.0) at 100°C (28), and oxidized with hydrogen peroxide. Oxidized CuCl extracts were separated by TLC (24). The distribution of the radioactivity on the developed TLC plates was analyzed by the Dickson program. For each analysis, 10,000 events were recorded.

RESULTS

Methylation of iAs\textsuperscript{III} Increases Cytotoxicity in NB4 Cells. We compared growth inhibition and cell death by treatment with iAs\textsuperscript{III}, iAs\textsuperscript{V}, and their monomethylated and dimethylated derivatives in NB4 cells at concentrations of 0.5, 1, and 2 μM for 1–3 days. MAS\textsuperscript{III}O treatment was more growth inhibitory (IC\textsubscript{50} = 0.3 μM) than iAs\textsuperscript{III}, whereas DMAS\textsuperscript{III}I was similar to iAs\textsuperscript{III} (IC\textsubscript{50} = 1.0 μM) at 3-day treatment (Fig. 1A). There was minimal mass of viability after treatment with 0.5 μM of iAs\textsuperscript{III}, DMAS\textsuperscript{III}I, and MAS\textsuperscript{III}O. However, 1 μM of MAS\textsuperscript{III}O caused 90% loss of viability of NB4 cells, whereas iAs\textsuperscript{III} and DMAS\textsuperscript{III}I remained at 15% (Fig. 1B). Treatment with up to 2 μM of pentavalent methylated arsenicals (MAS\textsuperscript{V} and DMAS\textsuperscript{V}) 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/H\textsubscript{11001}/PI/H\textsubscript{11002} assay was greatest after MAs\textsubscript{III}O treatment of NB4 cells. One M 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. 2 A). Similarly, K562 human leukemia cells are more growth inhibited (IC\textsubscript{50} 0.7\textsubscript{versus} 2.1 M) and undergo greater apoptosis (12.5\% versus 1.8\%) by 48 h of treatment with 1 \mu M 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. 2 B).

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...
Thus, these data demonstrate that degradation of PML-RAR in NB4 cells, and partially reformed PML nuclear bodies (Fig. 4B) microscopy using PML antibody demonstrated that iAs III treatment caused ~10-fold more H_2O_2 accumulation than iAs III (Table 1) and correlated with the extent of GPx inhibition. These data support the idea that MAs III O, like As III , induces apoptosis in APL cells by a H_2O_2-mediated pathway.

**MAs III O-induced Apoptosis Is Independent of PML-RARα Protein Degradation.** To evaluate the role of PML-RARα protein degradation in MAs III O-induced apoptosis, we measured PML-RARα levels by Western blot analysis and PML levels by immunocytochemistry using confocal microscopy. NB4 cells treated with 1 μM MAs III O for 24 h produced more apoptosis than treatment with 1 μM iAs III (Fig. 2A). However, iAs III but not MAs III O treatment degraded PML-RARα in NB4 cells after 24 h (Fig. 4A). Furthermore, confocal microscopy using PML antibody demonstrated that iAs III treatment but not MAs 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-RARα protein is not a requirement for the more effective MAs III O-induced apoptosis.

**PML-RARα protein degradation after iAs 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 III- but not MAs III O-treated NB4 cells undergo partial differentiation as measured by CD11b using FACS analysis.**

**MAs III O Is A More Potent Cytotoxic Agent in Human Lymphoma Cells.** Some lymphoma cells are also sensitive to low concentration of As_2O_3-induced apoptosis (30). The ability of MAs III O to induce growth inhibition and cell death was studied in two lymphoma cell lines: Jurkat cells, which are insensitive, and Namalwa cells, which are sensitive to As_2O_3-induced apoptosis. As shown in Fig. 5A, MAs III O was more growth inhibitory than iAs III in both Jurkat and Namalwa cells, because treatment with 1 μM iAs III resulted in <10%, whereas MAs III O 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 III O treatment (Fig. 5B).

CLL cells in primary cultures are minimally affected by treatment with 2 μM iAs III for 3 days. In contrast, 0.5 μM MAs 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 0.5 μM arsenite (MAs) and DMAs. DMAs was the major methylated metabolite 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).

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

Metabolism of iAsIII 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 [73As]iAsIII for 48 h (Table 3; Ref. 4). Radiolabeled metabolites of [73As]iAsIII 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 iAsIII concentration. U937 human leukemia cells like NB4 cells take up but do not methylate [73As]iAsIII (data not shown). In the individual and combined cultures containing HepG2 cells, [73As]iAsIII 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 iAsIII. In contrast, only 8.5% of iAs in the medium was taken up by NB4 cells. In the combined cultures exposed to 1 μM iAsIII 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 released into the medium by HepG2 cells when exposed to 1 μM arsenite.

Among all of the biologically relevant chemical forms of arsenic, the toxicity of MAs III O is a stronger cytotoxic agent than iAsIII in primary cultured CLL cells.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>As metabolites formed in HepG2 and leukemia cells incubated with 0.1 μM [73As] arsenite for 48 h (pmol As/culture, Mean ± SD, n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured cells</td>
<td>Medium</td>
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<tr>
<td></td>
<td>iAs</td>
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</tr>
<tr>
<td>HepG2 alone</td>
<td>128.5 ± 6.07</td>
</tr>
<tr>
<td>NB4 alone</td>
<td>183.4 ± 3.11</td>
</tr>
<tr>
<td>NB4 Plus HepG2</td>
<td>129.7 ± 3.30</td>
</tr>
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DISCUSSION

We established that NB4 cells can take up and accumulate methylated metabolites of iAs produced and released by HepG2 cells using.
MAsIII derivatives are the most potent enzyme inhibitor, as shown here for GPs (Fig. 3), and cytotoxin (reviewed in Ref. 32). In addition, MAsIII and DMAsIII 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 MAsII O and DMAsIII chemical precursors of methylated trivalent metabolites of iAsIII (Figs. 1 and 2). Our data indicate that the apoptosis-induction ability of MAsII O=DMAsIII>iAsIII. MAsII O treatment also inhibits growth and induces apoptosis to a greater extent than iAsIII. This suggests that the amount of MAsII O in cells may mediate the sensitivity to As2O3-induced apoptosis in malignant cells and normal tissues such as bone marrow and liver. The unexplained 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-arsenite 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 DMAsIII 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 MAsII O-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). MAsII O 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 MAsII O (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 MAsII O than iAsIII to degrade PML-RARα or PML. However, PML-RARα protein degradation follows iAsIII but not MAsII O treatment in NB4 cells (Fig. 4A). Moreover, iAsIII decreased abnormal PML nuclear body distribution and number as reported before (Fig. 4B), whereas neither MAsII O nor DMAsIII (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, MAsII O 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 vitro therapeutic contribution of MAsII O would be the induction of apoptosis, not differentiation. The contribution of hepatic derived MAsIII from iAsIII to therapeutic outcome remains to be determined. MAsII O 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 MAsII O in lymphoma cells (Fig. 5) and in CLL cells in primary culture (Table 2) supports the evaluation of As2O3 and MAsII O in additional clinical trials. MAsII O, 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 MAsII O by HepG2 cells support the use of iAsIII or MAsII O in the treatment of patients with hepatoma. There may be an increased potential for toxicity, particularly in liver with MAsII O treatment. Thus, animal studies are required to evaluate this derivative before future clinical trials.

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