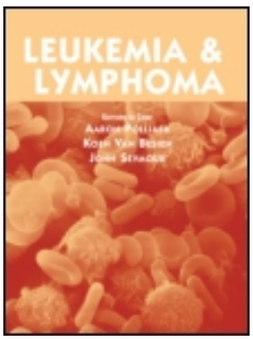

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Arsenic Trioxide: Acute Promyelocytic Leukemia and Beyond

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Arsenic containing treatments have a history of over two millenniums. Recently, arsenic trioxide (As_2O_3) has been introduced into the treatment of both *de novo* and relapsed acute promyelocytic leukemia (APL), with remarkable clinical success. Several investigations using both freshly isolated APL blast cells as well as APL-derived tumor cell lines have shown that the main mechanism by which As_2O_3 exerts its antileukemic activity in APL is induction of apoptosis in the leukemic cell population. Recently, it has become evident that the apoptotic effects of As_2O_3 are not restricted to APL cells but may also be observed in malignant cells of non-APL origin. In the present review, history, current clinical use as well as future perspectives of As_2O_3 therapy in both hematologic and solid malignancies are discussed, with special emphasis being put on the potential future role of As_2O_3 in the treatment of non-APL tumors. Of particular importance, enhancing agents suited to increase As_2O_3-sensitivity in less sensitive tumors (e.g. ascorbic acid) are also addressed.

Keywords: Arsenic trioxide; Acute promyelocytic leukemia; Apoptosis; Reactive oxygen species; Glutathione redox system

HISTORY

Arsenic containing treatments have a history of over two millenniums. Hippocrates and Dioscorides used arsenic sulfides (As_2S_2, As_2S_3) for the treatment of ulcers and as a depilatory. Furthermore, attempts to use arsenic against infectious diseases were made against the plague, against malaria and, following Ehrlich's discovery of the organic arsenical salvarsan, successfully against syphilis. Today, melarsoprol, another organical arsenic product, is used for the meningoencephalic phase of trypanosomiasis. Until recently, this has been the only indication for arsenic use in modern day medicine [1,2].

In the 18th century, Fowler's solution, a solution of arsenic trioxide (As_2O_3) in potassium bicarbonate, was used empirically to treat a variety of non-malignant and malignant diseases, including Hodgkin's disease and leukemia. In 1878, the ability of As_2O_3 to reduce leukocyte counts was first described in two normal individuals and a patient affected with CML, in whom a dramatic decline in white blood cell counts was seen following As_2O_3 treatment [3]. After As_2O_3 therapy had been replaced by radiation therapy in the early 20th century, a report in 1931 where 9 of 10 patients with CML responded to As_2O_3 therapy [4] led to a brief resurgence of arsenic use in leukemia treatment. However, long-term As_2O_3 use resulted in chronic arsenic poisoning [5] and led, together with the introduction of cytotoxic chemotherapy, to a progressive decline of arsenic use in Western Medicine. As_2O_3 was not rediscovered until the 1970s when Chinese investigators, looking back to a long practice of As_2O_3 use in Traditional Chinese Medicine, formally introduced "Ai ling-1", a solution containing herbal extracts combined with crude As_2O_3, into the treatment of patients with acute promyelocytic leukemia (APL) [6].

CURRENT CLINICAL USE OF ARSENIC TRIOXIDE IN APL

APL represents approximately 10–15% of acute myeloid leukemias. It is characterized by abnormal heavily granulated promyelocytes in the bone marrow and peripheral blood as well as a severe coagulopathy attributable to the release of procoagulant factors from the leukemic cells with consecutive disseminated intravascular coagulation and hyperfibrinolysis. Furthermore, APL cells

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characteristically display a reciprocal translocation of chromosomes 15 and 17 which disrupts the promyelocytic leukemia gene (PML) on chromosome 15 and the retinoic acid receptor-α gene (RARα) on chromosome 17 and leads to formation of two chimeric proteins, PML/RARα and RARα/PML. The PML/RARα fusion transcript can be found in almost all patients with the t(15;17) translocation, whereas the RARα/PML protein is detected in approximately two-thirds of patients [7–10].

Before the 1990s, patients with APL were treated with anthracyclines combined with cytosine arabinoside for induction, followed by additional cycles of chemotherapy for consolidation and/or maintenance. This therapeutic approach led to complete remissions (CRs) in approximately 60–80% of patients, with 5-year survival rates of 20–30%. In the early 1990s, all-trans retinoic acid (ATRA) was introduced into the treatment of APL, with an approximately two-fold improvement of overall and disease-free survival rates [11]. However, despite these advances in the treatment of APL, 20–30% of patients with APL relapse and die from the disease, unless rescued by bone marrow transplantation (which, although potentially curative, so far remains reserved for younger patients with an available donor) [10].

In 1992, Sun *et al.* reported promising results of 32 cases of APL treated with As_2O_3: 50% of the patients survived more than 5 years, without having received any anthracycline- or ATRA-based therapy [12]. In a trial performed at the Shanghai Second Medical University, CRs could be achieved in 14 of 15 investigated patients that had relapsed after prior treatment with ATRA and/or conventional chemotherapy (median duration of treatment: 38 days, range 28–44 days) [13]. The treatment regimen consisted of 10 mg As_2O_3 daily, infused over a 2–3 h period. In addition, a multicenter trial performed by Niu *et al.* reported remarkable efficacy of As_2O_3 monotherapy in 7 newly diagnosed and 31 relapsed cases of APL: CR rates were 85.7% (6 out of 7 cases) in newly diagnosed patients and 83.9% (26 out of 31 cases) in relapsed patients, with the median duration to achieve CR amounting to 35 and 30 days, respectively. Of particular interest, long-term As_2O_3 therapy was followed by molecular remissions in several patients [14].

In the Western population, clinical efficacy of As_2O_3 was first proved in 1998 in a study performed by Soignet *et al.* at the Memorial Sloan-Kettering Cancer Center and the Cornell University Medical College, New York [15]. In this study, 11 of 12 (92%) patients treated with a median daily dose of 0.16 mg/kg As_2O_3 (range 0.06–0.20 mg/kg) showed a CR. The median treatment duration was 33 days (range 12–39 days) and CR was attained by a median of 47 days (range 24–83 days). The duration of remission amounted to a median of more than 5 months (1–9 months). In addition, 8 of 11 patients who had initially tested positive for the PML/RARα translocation by RT-PCR became negative following treatment.

Adverse Effects

Adverse effects are a very sensible issue when dealing with As_2O_3. As_2O_3 has been known to be toxic for centuries—in fact, odor- and taste-lacking properties have made it an attractive poison. Importantly however, even though precancerous effects of arsenicals after environmental exposure have been well documented in the literature, long-term follow-up of 62 As_2O_3-treated pediatric patients with APL did not show an increase in secondary malignancies [16]. Adverse effects observed in several clinical trials with As_2O_3 comprised peripheral neuropathy (which resolved spontaneously after the end of treatment), leukocytosis during the induction phase and the APL differentiation syndrome which was observed in approximately 30% of cases. The APL differentiation syndrome is clinically identical to the retinoid acid syndrome and can be effectively treated with dexamethasone if treatment is initiated at the first sign or symptom [10]. Hepatotoxicity may also be encountered during treatment with As_2O_3: Niu *et al.* described hepatotoxic effects of As_2O_3 treatment in 7 of 11 patients with *de novo* APL, two of whom eventually died from hepatic failure [14]. In 15 of 47 relapsed patients included in the same study, mild hepatic damage was seen. Other trials with As_2O_3 found little and if so, only moderate hepatotoxicity [13,15]. A commonly encountered problem upon As_2O_3 treatment are abnormalities on ECG: These comprise QT prolongation, torsade-de-pointes arrhythmia and ventricular tachycardia. The management of QT prolongation consists of maintaining adequate serum potassium and magnesium levels (>4.0 mEq/dl and >1.8 mg/dl, respectively) and corrective actions if the QTc exceeds >500 ms [10].

Taken together, it has to be stated that the adverse effects of As_2O_3 therapy seem justifiable in comparison to other leukemia treatments such as cytotoxic chemotherapy. Particularly, As_2O_3 therapy does not induce significant bone marrow suppression, which in concert with its high efficacy at inducing CR in patients with APL, makes it a highly attractive agent in the treatment of patients with both *de novo* and relapsed APL.

MECHANISM OF ACTION

The promising clinical results of As_2O_3 in both *de novo* and relapsed APL led to *in vitro* investigations aimed at elucidating the cellular and molecular effects underlying the clinical efficacy of As_2O_3 in the treatment of APL. Although the exact mechanism by which As_2O_3 exerts its antileukemic effect remains unknown, several mechanisms have been implicated to contribute to the clinical efficacy of As_2O_3 *in vivo*: These include inhibition of proliferation, degradation of the APL-specific PML/RARα fusion transcript allowing partial differentiation of APL blasts, inhibition of GTP-dependent polymerization and microtubule formation as well as

inhibition of angiogenesis [15,17–20]. Finally—and may be most importantly—As_2O_3 has been shown to be a powerful apoptosis-inducing agent through its modulating effects on pro- and antiapoptotic molecules such as Bcl-2 and Bax as well as its ability to induce intracellular accumulation of reactive oxygen species (ROS) with consecutive mitochondrial membrane collapse and caspase activation [21–24].

Degradation of PML/RARα and Partial Differentiation of APL Blasts

In APL cells, As_2O_3 has been shown to target onto nuclear bodies and to induce degradation of the PML/RARα fusion transcript responsible for the maturation block at the promyelocyte stage of myeloid differentiation typically observed in APL [17,18]. Serially performed immunophenotypic studies in APL patients treated with As_2O_3 were able to correlate the degradation of PML/RARα with a decrease in the proportion of leukemic cells expressing the primitive myeloid antigen CD33 and a simultaneous increase in the proportion of leukemic cells expressing CD11b, a typical marker of more mature myeloid elements. Furthermore, As_2O_3 therapy was also associated with the appearance of a population of double-positive cells expressing both the CD33 and CD11b antigen [15]. Thus, it has been suggested that one of the mechanisms by which As_2O_3 induces clinical remission in APL is partial differentiation of the leukemic cell population, similar to the effects seen upon treatment of APL with ATRA.

Induction of Apoptosis: Modulation of Bcl-2 and Bax, Generation of Intracellular ROS

Bcl-2 and Bax are two integral mitochondrial membrane proteins with crucial functions for apoptosis-regulation. Bcl-2 is antiapoptotic and can protect cells from apoptosis induced by a variety of agents. Bax, on the contrary, has proapoptotic functions. The main mechanism by which Bcl-2 protects from apoptosis is heterodimerization with Bax, whereby it neutralizes the proapoptotic effects of Bax. *In vitro* studies in APL-derived NB4 cells and various other leukemic cell lines have shown that As_2O_3, at clinically achievable concentrations of 1–2 μmol/l, is able to downregulate Bcl-2 and thus shift the intracellular balance of pro- and antiapoptotic signals towards apoptosis with consecutive apoptotic cell death [21–23].

In addition, Jing *et al.* were able to demonstrate that As_2O_3 also has crucial implications for the intracellular redox state of malignant cells, i.e. by generating increased amounts of intracellular ROS [24]. Generation of intracellular ROS causes loss of the outer mitochondrial membrane potential with subsequent changes in mitochondrial membrane permeability and finally release of proapoptotic messenger molecules. It could be shown that As_2O_3 inhibits the ROS-metabolizing enzyme glutathione peroxidase, thereby leading to increased levels of intracellular hydrogen peroxide (H_2O_2) [24]. The accumulating H_2O_2 causes a decrease in the mitochondrial membrane potential, followed by the release of cytochrome c from the mitochondria, activation of the caspase cascade and finally apoptotic cell death.

Inhibition of GTP-dependent Polymerization and Microtubule Formation

Using a variety of different myeloid cell lines, Ming *et al.* were able to show that As_2O_3 causes mitotic arrest of malignant cells, similar to the effects of antitubulin drugs [19]. In an *in vitro* microtubule assembly assay they could demonstrate that prior treatment of monomeric tubulin with As_2O_3 markedly inhibits GTP-induced polymerization and microtubule formation, since As_2O_3 acts as a non-competitive inhibitor of GTP binding to tubulin: As_2O_3 binds to two cysteine residues in tubulin, thereby blocking the binding site for GTP which consecutively disrupts the normal dynamics of microtubules during mitosis and finally results in mitotic arrest. As seen with other microtubule inhibitors, a cascade of genes for programmed cell death is activated subsequently, eventually leading to apoptotic cell death.

Inhibition of Angiogenesis

Recent data suggest that the endothelium and angiogenesis play a pivotal role in the proliferation of hematological tumors. In particular, it could be shown that activated endothelial cells are able to release cytokines which stimulate leukemic cell growth. On the other hand, leukemic cells may release endothelial growth factors such as vascular endothelial growth factor (VEGF) and, in turn, cause activation and proliferation of the endothelium. Recently, Roboz *et al.* have been able to show that As_2O_3 may interrupt this reciprocal positive feedback loop between the leukemic cells and the endothelium by directly influencing both cell types: They were able to demonstrate that As_2O_3 inhibits VEGF production by leukemic cells, prevents capillary tubule and branch formation by endothelial cells and, finally, induces apoptosis in both leukemic blasts as well as rapidly proliferating endothelial cells [20]. It has thus been concluded that inhibition of angiogenesis may be another important mechanism of action of As_2O_3.

FUTURE PERSPECTIVES

Owing to its multifactorial and universal mechanism of action (ROS-dependent induction of apoptosis, inhibition of microtubule function, inhibition of angiogenesis (Fig. 1)) it has been speculated by several groups that the apoptotic effects of As_2O_3 might not be restricted to APL cells but may also be observed in other tumors. Several *in vitro* studies have therefore been performed in non-APL cells, with the aim of elucidating the potential

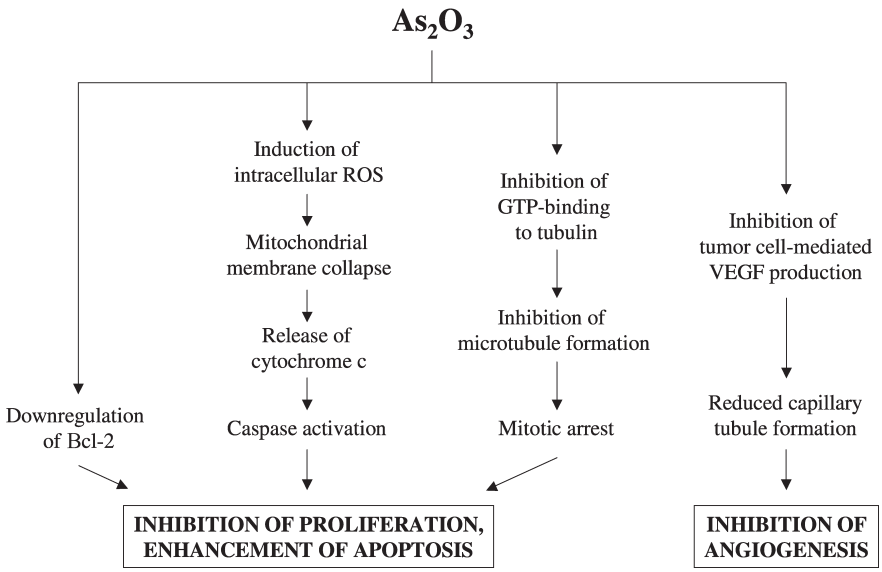

FIGURE 1 Mechansim of action of As_2O_3: ROS-dependent induction of epoptosis, inhibition of microtubule function and inhibition of ongiogenesis. ROS = reactive oxygen species; VEGF = vascular endothelial growth factor.

role of As_2O_3 in the treatment of malignancies other than APL [22,23,25–30]. These investigations have confirmed that, indeed, the apoptotic effect of As_2O_3 is not specific for APL cells but may also be observed in other malignant cells such as non-APL acute myeloid leukemia cells, chronic myeloid leukemia cells, myeloma cells as well as various solid tumor cells. However, it also became evident that non-APL tumor cells may display an up to 10-fold lower sensitivity to As_2O_3 as compared to APL cells [31]. In an attempt to elucidate the pathophysiologic processes underlying the variable sensitivity of malignant cells to As_2O_3, Dai *et al.* were able to demonstrate that the glutathione redox system plays a decisive role for As_2O_3-induced apoptosis: they showed that the sensitivity of a given cell to As_2O_3 inversely correlates with the intracellular content of reduced glutathione (GSH): That is, cells with a low intracellular GSH content (such as APL-derived NB4 cells) are sensitive to clinically achievable concentrations of 1–2 μmol/l As_2O_3, whereas cells with a high GSH content (such as acute myeloid leukemia-derived HL60 cells which have an approximately 3-fold higher intracellular GSH content than NB4 cells) are As_2O_3-resistant [32].

As a result, attempts have been made to render less sensitive tumor cells susceptible to As_2O_3-mediated apoptosis by means of modulation of the GSH redox system, with the intention to expand the therapeutic spectrum of As_2O_3 onto a broader variety of hematologic as well as solid tumors. Indeed, it could be demonstrated that buthionine sulphoxide, which reduces intracellular GSH by means of inhibition of γ-glutamylcysteine synthetase, can render previously As_2O_3-resistant cancer cells sensitive to 1 μmol/l As_2O_3. On the other hand, experimental upmodulation of intracellular GSH by means of *N*-acetylcysteine treatment was able to prevent As_2O_3-mediated apoptosis in the previously sensitive APL cell line NB4 [32]. Other substances shown to increase As_2O_3-sensitivity in less sensitive tumor cells are mercaptosuccinic acid and aminotriazol, two potent inhibitors of the major H_2O_2-scavenging enzymes glutathione peroxidase and catalase, respectively [24].

Of particular interest, several *in vitro* investigations led to the discovery of ascorbic acid as a highly promising substance to increase As_2O_3-sensitivity: In initial studies performed in HL60 cells it could be shown that ascorbic acid both decreases the intracellular GSH content as well as leads to increased intracellular H_2O_2 levels by means of autooxidation [32]. The dual effect of GSH-downmodulation and H_2O_2-upregulation leads to a significant synergistic effect with As_2O_3. In a mouse model, ascorbic acid enhanced the antilymphoma effect observed in response to As_2O_3 treatment, without additional cytotoxic side effects on normal tissues. Consecutively, in an *in vitro* study performed by our group, we were able to confirm the initial observations on ascorbic acid-mediated enhancement of As_2O_3-mediated apoptosis: Using several freshly isolated AML patient samples we could show a significant synergistic effect of ascorbic acid (125 μmol/l) on As_2O_3-mediated apoptosis [26]. This made us conclude that ascorbic acid might be a valuable substance to increase As_2O_3-sensitivity in AML patients who are intrinsically resistant to As_2O_3 as well as to overcome acquired As_2O_3 resistance in patients with APL.

Recently, it has been shown that ascorbic acid also synergizes with As_2O_3 in multiple myeloma cell lines as well as freshly isolated myeloma cells: Grad *et al.* were able to show that combined treatment of myeloma cells with ascorbic acid (100 μmol/l) potentiated As_2O_3-mediated cell death of several myeloma cell lines as well as freshly isolated myeloma cells [33]. Of particular importance, normal bone marrow cells displayed little sensitivity to combined As_2O_3/ascorbic acid treatment,

suggesting that the combination therapy may be selectively toxic to tumor cells while at the same time sparing normal hematopoietic cell populations.

The broad mechanism of action of As_2O_3 and the possibility to increase As_2O_3-sensitivity using enhancing agents such as ascorbic acid have evoked high interest in As_2O_3 as a new therapeutics for a variety of hematological as well as solid malignancies. Based on the promising *in vitro* results of As_2O_3 in non-APL tumor cells, several clinical studies of As_2O_3 in different hematologic as well as solid malignancies have thus been initiated, with the intention to evaluate the therapeutic potential of As_2O_3 in non-APL tumors. In one study performed in patients with relapsed or refractory multiple myeloma, ascorbic acid has been added to the study protocol in order to increase the efficacy of treatment (for a detailed review, see Ref. [34]).

In conclusion, As_2O_3 has emerged from being a promising treatment for APL to become a highly interesting experimental therapeutics for a wide variety of hematological as well as solid tumors. In our opinion, a highly interesting area of future As_2O_3-research will be the elucidation of other possible enhancing agents suited to augment the apoptotic effects of As_2O_3 in less sensitive cancer cells. Potential strategies include substances which reduce GSH and/or increase intracellular ROS production.

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