

The Other Side of Arsenic

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Abstract : Though Arsenic is a well known toxic heavy metal but some beneficiary functions of arsenic are discussed in this review.

Introduction:

Arsenic toxicity has been reported from many parts of the world. While Bangladesh and West Bengal (India) account for the most of the incidence, occasional reports from Mexico, Taiwan and mainland China have also appeared. A wide variety of pathological conditions have been observed in humans due to arsenic toxicity. The toxicities and side effects of arsenic compounds are well known. Based on specific tissue exposed and the time and degree of exposure, in experimental animals various syndromes and disorders can be observed. While exposure during pregnancy can result in impaired fetal growth or even fetal loss, prolonged and high dose exposure to arsenic can cause various types of cancers in lung, bladder, skin, renal and prostate gland. Severe gastrointestinal toxicities, diabetes, vascular changes, cardiac arrhythmias and death have also been reported. Arsenic and its methylated species are known cocarcinogens which act by facilitating/ promoting the induction of tumors of the skin, urinary bladder, and lung rather than directly inducing cell transformation and oncogenesis. As cocarcinogens, mechanisms include indirect effects such as DNA damaging genotoxicity by altered DNA methylation as well as inducing high levels of oxidative stress leading to altered cell proliferation and tumor promotion. Moreover, chronic exposure to arsenic can affect cell signaling pathways leading to deregulation of cell functions. Despite its toxicity, arsenic has been used in Chinese medicine for thousands of years in the form of refined preparations of realgar (As_4S_4) or orpiment (As_2S_3). Ayurvedic practice of Rasa Shastra describes herbal medicines combined with metals and gems, arsenic occupying an important position. Thus there seems to be another not much explored beneficial facet of arsenic. This article tries to summarize the recent advances in that direction.

Antineoplastic Properties: Although exposure to arsenic leads to development of a variety of malignancies, one form of arsenic, As_2O_3 , exhibits potent antitumour effects *in vivo* and *in vitro*. As_2O_3 has been approved by the FDA (American agency that approves new medicines) for the treatment of acute promyelocytic leukemia (PML) in humans which is unresponsive to conventional drugs like ATRA (all-trans-retinoic acid). Arsenic trioxide when tried alone has provided complete hematologic remission with minimal toxicity in 86% of APL patients. This is equal to any of the current methods of treating APL like for example the combination of all-trans retinoic acid (ATRA) and chemotherapy. Combinations of As_2O_3 with ATRA for the treatment of this leukemia are considered to be much more effective than arsenic trioxide alone. This raises the question why APL cells are very sensitive to arsenic containing compounds like As_2O_3 . This selectivity may be due to the fact that APL cells express the transmembrane transporter protein, 'aquaglyceroporin 9' (AQP9) at much higher levels in APL cells than in other leukemic cell types. This particular

aquaporin allows arsenic to pass through it thus enabling the metalloid to cross the cell membrane. The level expression seems correlate with the sensitivity of the malignant cells to arsenic. In this regard, it is worth noting that aquaglyceroporins AQP7 and AQP9 are present in normal cell types. Interestingly, AQP9 is primarily expressed in human lung, liver, and leukocytes at low concentration. The fact that AQP9 provides APL cancer cell specificity with high response rates suggests that if arsenic containing compounds could be targeted for specific delivery into cancer cells, then they would represent outstanding agents for killing these cells. Active investigations are underway to extend the use of As_2O_3 for the treatment of other hematological malignancies like multiple myeloma, acute myeloid leukemia and myelodysplastic syndromes. But associated toxicities like leukocytosis and cardiac arrhythmias associated with prolonged QT interval are frequently observed. Thus, appropriate precaution must be undertaken during As_2O_3 administration.

Arsenicals Lead To Cell Death: Current concepts regarding the mechanism by which As_2O_3 generates its potent cytotoxic and antitumour activities invitro and invivo are summarized below.

1. *Arsenic compounds frequently target elements and oncogenes selectively expressed in certain malignancies.:* Acute promyelocytic leukemia (APL) is associated with chromosomal translocations always involving the retinoic acid receptor alpha ($RAR\alpha$) gene situated on the chromosome 17. PML- $RAR\alpha$ (Promyelocytic leukemia – Retinoic acid receptor α) fusion protein in APL cells has the ability to impair cAMP signaling which is involved in combating oxidative stress. The unabated oxidative stress enhances the sensitivity of APL cells to As_2O_3 , which is particularly effective at killing APL cells in combination with retinoic acid. This potentiation is thought to be due to be the result of its ability to induce the relocalization and degradation of the nuclear body protein PML, as well as the degradation of PML- $RAR\alpha$ in APL cells which is required for leukemic precursor clearance. Cells expressing AML/MDS1/EV11 oncoprotein, a product of a fusion gene resulting from the t(3;21)(q26;q22) translocation, are sensitive to As_2O_3 and degraded at therapeutic concentrations of this drug. Arsenic also targets BCR-ABL oncoprotein via ubiquitination of key lysine residues, leading to its proteasomal degradation.

2. *Generation and modification of the redox regulation of reactive oxygen species is of high relevance in the induction of arsenic-mediated cell death:* Induction of apoptosis by As_2O_3 involves overproduction of ROS which is partially regulated by activation of NADPH oxidase and NO synthase isoenzymes. Arsenic has high affinity for sulphur-containing molecules such as reduced thiols. The resulting arsenic-thiol linkages are mainly responsible for the ability of arsenic to modulate the function of various key molecules, enzymes, and ion transporters inside cells. Inhibition of mitochondrial proteins regulating ROS generation by disulfide linkage of vicinal thiol groups often leads to increased production of ROS and induction of apoptotic signaling pathways. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the glycolytic enzyme abundantly found in all cells and especially blood cells and liver, is a major intracellular arsenate reductase requiring GSH, NAD, and glycolytic substrate. Given that the levels and specific activity of GAPDH is much

higher in malignant cells than in normal cells, this could contribute to the rapid reduction of As(V) species in their cytosol into more toxic As(III) forms. The multidrug resistance (MDR) protein MRP1/ABCC1 has been shown to transport As(III) out of cells as a tri-GSH conjugate $\text{As}(\text{GSH})_3$. Glutathione S-transferase (GST) probably facilitates the process. This is likely to be a part of the normal cellular resistance mechanisms against the cytotoxic effects of arsenic-based compounds. Thus, in order to promote antitumour effects of As_2O_3 invitro, pretreatment of cells with ascorbic acid or buthionine sulfoximine is done because they deplete intracellular GSH stores. But pretreatment of malignant cells with N-acetylcysteine leads to increase in cellular GSH levels and finally reversal of the effects of As_2O_3 . Thus, to enhance the sensitivity of cells to As_2O_3 , the cellular glutathione stores must be appropriately regulated. The cells are protected from stress by the thioredoxin system which includes thioredoxin, thioredoxin reductase and NADPH. Arsenic containing compounds block thioredoxin reductase and facilitate oxidative stress mediated proapoptotic effects.

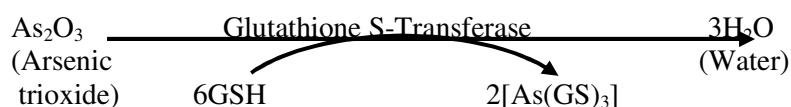


Figure 1: Glutathione S-transferase catalyses the formation of the tri-GSH conjugate with As(III) and confers resistance to arsenic by facilitating its active removal from the cell.

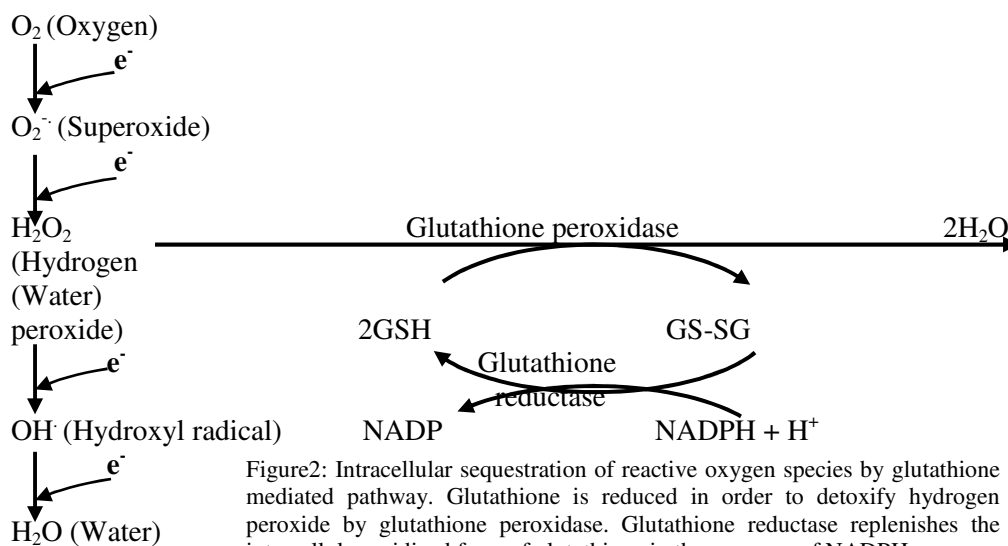


Figure2: Intracellular sequestration of reactive oxygen species by glutathione mediated pathway. Glutathione is reduced in order to detoxify hydrogen peroxide by glutathione peroxidase. Glutathione reductase replenishes the intracellular oxidized form of glutathione in the presence of NADPH.

3. Accumulation of H_2O_2 leads to decrease in the mitochondrial membrane potential, resulting in cytochrome c release and activation of the caspase cascade: Arsenite induces apoptosis by opening the MPTP (mitochondrial permeability transition pore) and by homodimerization of VDAC (Voltage dependent Anion Channel). This leads to abnormal in permeability of outer membrane of mitochondria and release of

cytochrome c. Experimental data indicates that VDAC contains critical Cys residues that can undergo intermolecular cross-links by reacting with arsenic. It has been established that the redox state of thiol reactive groups are important for activation of the mitochondrial permeability transition. As₂O₃ activates proapoptotic Bcl-2 family member Bax and induces its translocation from the cytosol to the mitochondria. Up regulation of expression of other proapoptotic proteins like Noxa, Bmf and Bim is also As₂O₃ mediated. Simultaneously, downregulation of other antiapoptotic proteins, including Bcl₂ (inhibitor of mitochondrial ROS generation), Bcl-1, Bcl-X_L and Mcl-1 is brought about arsenic administration. As₂O₃ dependent growth inhibition and apoptosis in cells of APL origin is dependent on activation of JNK pathway (c-Jun N-terminal kinase). An additional mechanism of inhibition of IκB kinase mediated via downregulation of NF-κB by As₂O₃ indicates its participation in induction of antitumour effects. It was recently shown that arsenite induces apoptotic response in melanoma cells by upregulating the expression of TNFα. Upon induction, these proapoptotic proteins bind to the outer mitochondrial membrane and induce cytochrome c release which in turn activates a caspase cascade. This mode of cell death is observed in various types of malignant cells like cells of APL origin, human T cell lymphotropic virus I-cell lines and primary adult T cell leukemia cells, multiple myeloma cells and different types of solid tumors cells. However, caspase-independent death pathways have also been reported to be activated by arsenic in myeloma cells and may mediate proapoptotic signals.

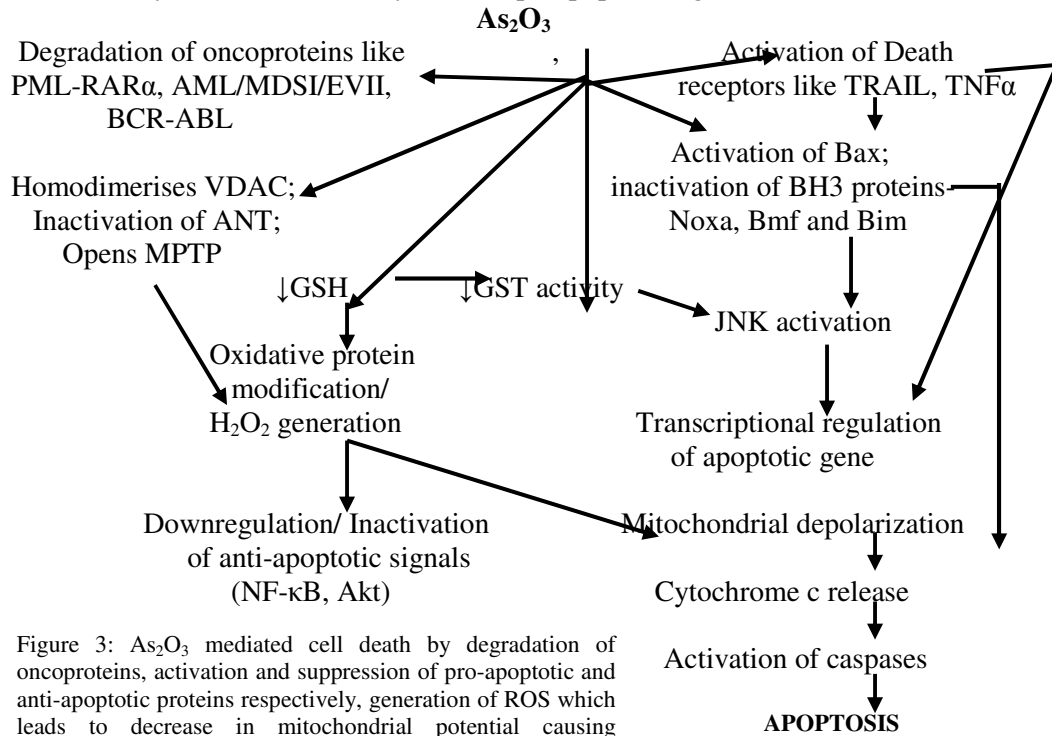


Figure 3: As₂O₃ mediated cell death by degradation of oncoproteins, activation and suppression of pro-apoptotic and anti-apoptotic proteins respectively, generation of ROS which leads to decrease in mitochondrial potential causing cytochrome c release and finally activation of the caspase cascade.

Arsenic inhibits JAK-STAT pathway. As₂O₃ suppresses activation of STAT1, STAT3 and STAT5 in AML cells and also JAK1 and JAK2 which phosphorylate STATs. There is also evidence that sodium arsenite inhibits interleukin6-dependent tyrosine phosphorylation of STAT3 in HepG3 cells via direct suppression of the tyrosine kinase JAK1. As₂O₃ and Hsp90 inhibitors act synergistically to inhibit STAT3 activity. Death receptors-induced apoptosis is also observed in cancer cells sensitive to As₂O₃. Studies reveal As₂O₃ sensitizes human glioma cells to TRAIL (TNF-related-apoptosis-inducing ligand)-induced apoptosis via DR5 upregulation.

4. *Pharmacological or molecular inhibition of certain cell signaling pathways is required to enhance As₂O₃ dependent generation of growth inhibitory and proapoptotic responses:* p38-MAPK pathway acts as a negative feedback regulator system to control induction of As₂O₃ responses in malignant hematopoietic cells and solid tumor cells. Recent studies have demonstrated that MKK3 and MKK6 are the upstream kinases that regulate arsenic dependent reengagement of p38. Two other downstream effectors of p38-MAPK, the kinases Mnk1 and Mnk2, are also activated in an As₂O₃-inducible manner and regulate phosphorylation of eukaryotic initiation factor 4at Ser-209. Other works have demonstrated pharmacological inhibition of p38 promotes As₂O₃ dependent-leukemic cell differentiation of APL and apoptosis in multiple myeloma cells. Similarly, MEK (mitogen-activated protein-kinase/extracellular signal regulated kinase kinase)-ERK pathway also negatively controls generation of antitumour responses by As₂O₃. Antileukemic responses of As₂O₃ is inhibited by MSK1 (mitogen and stress activated kinase 1) activation in response to stress. It is a common effector kinase for p38 and ERK MAPK pathways and mediates immediate-early gene expression. Efforts are in progress presently to identify specific down-stream MAPK-induced signals that suppress As₂O₃ responses so that specific less toxic drugs inhibitors could be designed and used in combination with As₂O₃ to enhance As₂O₃ dependent suppressive responses in leukemic lines and primary leukemic progenitors from AML patients. Akt-mTOR signaling cascade plays a critical role in the control of mRNA translation in mammalian cells and mediates important biological responses. Recent work demonstrated that Akt, mTOR and downstream effectors are activated in As₂O₃-treated leukemic cells.

A Novel Antineoplastic Agent: A novel approach to potentiate the effects of arsenic is to use knock out cells for different elements of mTOR pathway or small interfering RNA mediated knock-down of mTOR effectors. One of such compounds, the novel glutathionyl peptide trivalent arsenic-containing compound para 4-[N-(S-glutathionylacetyl)amino]phenylarsenoxide (p-GSAO) shows promise as a novel antineoplastic drug and is now in clinical trials. p-GSAO, inactivates (Adenine Nucleotide Transporter) ANT-mediated ATP/ADP transport and triggers Ca²⁺-dependent MPTP opening by crosslinking the critical Cys residues of ANT. This leads to increased production of cellular ROS, ATP depletion, mitochondrial depolarization, and apoptosis of angiogenic endothelial cells and inhibition of tumor growth in mice with no apparent toxicity. Tumor cells export p-GSAO much more efficiently than endothelial cells because they have higher MRP1 or MRP2 activity and cellular glutathione levels and this may explain why p-GSAO is not highly

effective at inhibiting tumor cell growth in vivo. In addition, the greater water soluble properties of p-GSAO than other arsenic containing compounds, particularly organic species should help to retain p-GSAO in the intravascular system where it is more likely to affect endothelial cells and inhibit tumor angiogenesis.

Conclusion

Our understanding of how arsenic mediates its various biological effects have expanded in recent years. But the usefulness of the antitumour properties of arsenicals has to be yet proved. Selective suppression of the negative regulators of the As_2O_3 in malignant cells may pave way to the development of newer antineoplastic drugs. Sensitivity or resistance to the actions of arsenic-containing compounds on cancer cells and normal cells depends on the levels of transport systems for their uptake or efflux from the cells as well as their redox defense mechanisms. Hence, development of a combination of selective delivery and retention provides the necessary targeting of arsenic-containing compounds to tumors and provides scope for additional modifications to be made to enhance the antineoplastic activity of arsenic-containing compounds, given their range of actions and efficiency in killing cancer cells.

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