The two opposite facets of arsenic: toxic and anticancer drug

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ABSTRACT
Arsenic compounds have been known and used for centuries but their effects in living organisms still represent a large unknown. Arsenic compounds have paradoxical effects: they are threatening to human health, especially upon long-term exposure that can induce the development of cancer; however, they are used as drugs against cancer. This review focuses on the effects shown by clinically and environmentally relevant arsenic compounds in living organisms with a focus on the calcium–apoptosis link.
1. HUMAN EXPOSURE TO ARSENIC AND HEALTH EFFECTS

Arsenic is a ubiquitous element found in air, water and soil. The contamination of drinking water with arsenic, that was documented all over the world, could be the result of natural sources and of human activity such as mining, industry or agriculture. In industry, arsenic is used for producing paints, fungicides, insecticides, herbicides, wood preservatives, cotton desiccants, semiconductors, light emitting diodes, lasers, and a variety of transistors. Additionally, arsenic has been used over time in agriculture as a pesticide. Thus, due to the globalisation of industry and agriculture respectively, and especially import-export activity, arsenic exposure no longer represents a local concern for regions where it is naturally occurring but can be introduced in other regions where arsenic was previously absent. Thus, arsenic represents a problem with local and global health perspectives.

Millions of people all over the world are affected by arsenic exposure, especially in the regions where drinking water is contaminated making arsenic toxicity is a worldwide health problem. Chronic exposure to arsenic correlates with the occurrence of several illnesses: reproductive defects, neurological and behavioural disorders, cardiovascular conditions, haematological diseases, diabetes, dermal changes, hearing loss, fibrosis of the liver and lung, renal disease, blackfoot disease, and several types of cancers including skin cancer, as shown in numerous epidemiological studies. How arsenic influences the occurrences of these diseases is still not completely understood, as numerous specific relationships between experimental and human exposure to arsenic are yet to be established.

Before the year 2001, the Environment Protection Agency (USA) allowed a so-called “permissible” level of arsenic in drinking water of 50 ppb. But after the year 2001 the Environment Protection Agency (USA) further reduced the permitted arsenic concentration to 10 ppb, 1/10 of what was previously acceptable. Hence, this arsenic concentration might not be sufficiently low to avoid arsenic toxicity and carcinogenity upon exposure of humans to drinking water.

In previous times, arsenic was constituent in cosmetics as well as in paints (e.g. pigment in “Paris green”). Furthermore, because arsenic compounds are tasteless and odourless, they played an important role in political theatre, being used as a poison weapon. For example, debates over the cause of Napoleon Bonaparte’s death often center on whether he was intentionally or accidentally poisoned with arsenic. In 1960, analyses of of Napoleon’s authenticated heirs confirmed that Napoleon suffered from chronic arsenic poisoning on the island of St. Helena. Timeline correlation of his clinical symptomatology of the preceding 4 months, as reported in the written diaries of his exiled companions, further supports the effect of fluctuating, elevated toxic levels of arsenic on his health. The assassination of Napoleon included both a cosmetic and lethal phase. The cosmetic phase consisted of arsenic poisoning over time to weaken Napoleon, making the associated debility appear to be a natural illness. When the lethal phase was carried out, Napoleon was given Calomel (HgCl), a cathartic, and a popular orange-flavored drink called orgeat, which was flavored with the oil of bitter almonds that form the lethal mercury cyanide.

Accidental death caused upon use of arsenic containing paints or of coal fires have also been documented. In the first case, a fungus (Scopulariopsis breviculis) was able to metabolise arsenic from the wallpaper, resulting in a volatile arsenic compounds (a mixture of arsine, dimethyl and trimethyl arsine). In the second case, the use of coal fires emitted hydrogen, which formed the toxic gas arsine due to the combination of the gas for lighting and arsenic found in paint “Paris green”. Fortunately, those sources of human exposure are today no longer of concern, while other sources, especially drinking water, still remain a problem.

2. MEDICAL USE OF ARSENIC

Arsenic has paradoxal effects. Arsenic compounds are potent toxins and carcinogens, but they have also been used medically for over 2000 years and are still used in diverse treatments (e.g. leukaemia, leishmaniosis, trypanosomiasis). Medical treatments with arsenic were used in antiquity (e.g. Greek physicians - Hippocrates), while during the nineteenth century, Fowler’s solution (1% arsenic trioxide) was widely used for leukaemia, skin conditions (psoriasis, dermatitis herpetiformis, and eczema), stomatitis and gingivitis in infants, and Vincent’s anginas, as a health tonic. Nevertheless, long-term use of Fowler’s solution caused haemangiosarcoma, angiosarcoma of the liver and nasopharyngeal carcinoma. Arsenic was the primary treatment for syphilis until World War II; (arsphenamine, neoaarsphenamine- 30%) and some protozoan infections.
Also in traditional Chinese medicine, arsenous acid or arsenic trioxide (As$_2$O$_3$) was often used as a devitalizing agent to treat tooth marrow disease, as well as psoriasis, syphilis, and rheumatosis. In the 1970s, As$_2$O$_3$ was introduced into the treatment of acute promyelocytic leukemia (APL) and showed large success in China. The clinical complete remission rate with As$_2$O$_3$ treatment (10 mg/d, intravenous infusion for 28 to 60 days) was in the range from 65.6% to 84%. As$_2$O$_3$ is now widely used to induce remission in patients with APL based on its mechanism of induction of apoptosis specifically in tumour cells.

3. MANIFESTATION OF ARSENIC INTOXICATIONS

In humans, the absorption of arsenic occurs mainly in the small intestine after ingestion, but also occurs with skin contact and inhalation. Arsenic exerts general toxicity inactivating enzymes involved in cellular energy pathways as well as in DNA synthesis and repair. No established treatment is available to handle chronic arsenic poisoning. Although antioxidants might be helpful, their benefit are not fully proven. The prevention of arsenic exposure, including the attempt to reduce arsenic ingestion from drinking water and food (e.g. alternative water supplies), is currently an important aspect of health management.

3.1 Acute arsenic poisoning

Acute arsenic intoxications show clinical manifestations such as: nausea, vomiting, colicky abdominal pain, profuse watery diarrhoea, excessive salivation, acute psychosis, toxic cardiomyopathy, haematological abnormalities, renal failure, respiratory failure, pulmonary oedema. Neurological manifestations could also appear as peripheral neuropathy or encephalopathy. Recently used indicators of poisoning include checking for the presence of arsenic in urine approximately 1–2 days after arsenic intake took place. Furthermore, analyses of blood, urine, and hair samples are used to quantify and monitor arsenic exposure: levels between 0.1 and 0.5 mg/kg on a hair sample indicate chronic poisoning, while 1.0 to 3.0 mg/kg indicates acute poisoning. Other biomarkers of arsenic exposure are being explored and include the analysis of urinary porphyrin levels and blood metallothionein levels.

3.2 Chronic arsenic poisoning

Chronic arsenic toxicity manifests in all body systems. Diagnosis relies basically on clinical and laboratory criteria. However, the cutaneous manifestations (melanosis, keratosis, and cutaneous cancers) are important diagnostic tool as well. While arsenic accumulates in the body (liver, kidneys, heart and lungs, muscles, nervous system, gastrointestinal tract, spleen, and lungs), it is deposited in keratin-rich tissues (nails, hair, and skin). The “Mee’s lines” are marker for arsenic intoxication because they appear after arsenic exposure in the fingernails and toenails. Dermatological changes are commonly expressed as hyper-pigmentation and keratoses. Upon chronic exposure to arsenic the risk of cardiovascular disease, peripheral vascular disease, diabetes mellitus, and neuropenia are significantly increased. The most important consequence of long time arsenic exposure is a malignant transformation of the cells (lymphoma as well as with skin, lung, liver, kidney, bladder, nasal cavity, bone, liver, larynx, colon and stomach-cancer).

Guo et al. analysed cancer registry data (1980–1987) of tumours of the bladder and kidney in Taiwan and reported that high arsenic levels in drinking water were associated with cell carcinomas of the bladder, kidney, ureter, urethral cancers (in males and females), and adenocarcinomas of the bladder in males. Effective treatment of chronic arsenic toxicity is not yet available but the so-called ‘on-specific therapies’ (e.g. keratolytics for hyperkeratosis) are applied and serve as palliative measures. The persons affected must be followed up at regular intervals to detect the earliest onset of cancers.

3.3 Arsenic neurotoxicity

The nervous system is an important target of arsenic. Neurological effects of arsenic may develop within a few hours after ingestion, but are usually observed 2–8 weeks after exposure. Neurological effects usually manifest as symmetrical sensorimotor neuropathy, often resembling the Guillain-Barré syndrome. The predominant clinical features of neuropathy are paresthesias, numbness and pain, particularly in the soles of the feet. Electrophysiological studies performed on
patients with arsenic-induced neuropathy have revealed a reduced nerve conduction velocity, typical of those seen in axonal degeneration.\textsuperscript{5,6}

Arsenic induces peripheral neuropathy, changes in behaviour, confusion, and memory loss. Cognitive impairment was reported in workers after 14–18 months of exposure, but mental function returned to normal after withdrawal of the arsenic exposure. An increased prevalence of cerebrovascular disease was observed in a large study of 8102 men and women who experienced long-term arsenic exposure in drinking water.\textsuperscript{1,3,40–42} Most of the adverse effects of arsenic are caused by inactivated enzymes in the cellular energy pathway, whereby arsenic reacts with the thiol groups of proteins and enzymes and inhibits their catalytic activity.\textsuperscript{5} Furthermore, arsenic-induced neurotoxicity causes changes in cytoskeletal protein composition and hyperphosphorylation, which lead to disorganization of the cytoskeletal framework.\textsuperscript{5}

4. MOLECULAR MECHANISMS OF ARSENIC INTERACTION WITH LIVING CELLS

The mechanisms of arsenic interaction with living cells are not fully understood. After intake, arsenic is biomethylated resulting in organic trivalent and pentavalent arsenic compounds. For a long time, the biotransformation of arsenic was believed to be a detoxification process, because the pentavalent organic forms of arsenic showed little or no toxic effects \textit{in vitro}. Today, the arsenic methylation is regarded as a toxification process. The reasons are that the trivalent organic forms of arsenic, which are produced intracellularly (upon biotransformation), are more toxic than the inorganic arsenic (for review see \textsuperscript{1,4,6,43,2} Only little arsenic is taken up in the \textit{in vitro} cell models but pentavalent forms of organic arsenic have shown clastogenic effects after forced uptake.\textsuperscript{4,6,44}

Inorganic arsenic has two biological important oxidation states: As(V) (arsenate) and As(III) (arsenite) that are chemically similar to required nutrients.\textsuperscript{4,45} Arsenate resembles phosphate and is a competitive inhibitor of many phosphate-utilizing enzymes and thus taken up by phosphate transport systems.\textsuperscript{45} In contrast, at physiological pH, the form of arsenite is As(OH)(3), which resembles organic molecules such as glycerol. Consequently, arsenite is taken into cells by aquaglyceroporin channels.\textsuperscript{45} Arsenic efflux systems are found in nearly every organism and evolved to rid cells of this toxic metalloid. These efflux systems include members of the multidrug resistance protein family and the bacterial exchangers Acr3 and ArsB. ArsB can also be a subunit of the ArsABAs(III)-translocating ATPase, an ATP-driven efflux pump.\textsuperscript{45} The ArsD metallochaperone binds cytosolic As(III) and transfers it to the ArsA subunit of the efflux pump.\textsuperscript{45}

Arsenic interferes with physiological processes by replacing physiological metals (e.g. zinc, selenium) from their binding sites in molecules.\textsuperscript{5,10,46} Arsenic contributes to reactive oxygen species (ROS) production, thus damaging DNA, lipids, or proteins as well as activating oncoproteins (e.g. c-myc). Arsenic interferes with signalling transduction pathways related to cell growth, cell proliferation, and apoptosis. Moreover, arsenic may act as a co-carcinogen, tumour promoter, or tumour progressor, under certain circumstances inducing cancer development.\textsuperscript{1,3,7,10,13,15,46,47}

Different possible modes of action of arsenic-induced carcinogenesis have been proposed: chromosomal damage, oxidative stress, modification of gene expression, modulation of DNA repair or DNA methylation and interactions with growth factors or cell proliferation. Arsenic-induced carcinogenesis could also be a result of promotion/progression, gene amplification, suppression of p53, as well as global DNA hypomethylation or malignant transformation.\textsuperscript{10,33,48,49} Arsenic induces the formation of oxidized lipids, which in turn generate several bioactive molecules (ROS, peroxides and isoprostanes); these molecules represent aspects of chronic and acute arsenic exposures in the etiology of arsenic induced diseases.\textsuperscript{30}

Arsenic is a non-mutagenic human carcinogen that induces tumors through a not completely understood mechanism. Various genotoxic effects of arsenic are documented in humans: chromosomal aberration and increased frequency of micronuclei in different cell types have been found to be significant increased.\textsuperscript{11} These effects can be the result of ROS formation, DNA repair deficiency, perturbation of methylation of promoter region of p53 and p16 genes, and genomic methylation alteration etc.\textsuperscript{11}

New indications suggest that the carcinogenicity of arsenic results from epigenetic changes, particularly in DNA methylation. Changes in gene methylation status, have been proposed to activate oncogene expression or silence tumor suppressor genes, thus influencing cell transformation.\textsuperscript{11,50} Studies show that arsenic exposure is associated with both hypo- and hyper-methylation at various genetic loci \textit{in vivo} or \textit{in vitro}, however it is not clear whether the changes induced by arsenic are
causally involved in the transformation process or a result of the altered physiology of cancer cells.\textsuperscript{11,50} Furthermore, deregulation of miRNAs might be an important epigenetic mechanism that contributes to arsenic induced carcinogens.\textsuperscript{51} Several miRNAs that controlled apoptosis related processes, were deregulated upon arsenic treatment.\textsuperscript{51} Nevertheless more research is necessary to discover the role of miRNAs in arsenic induced carcinogenesis.

Furthermore, arsenic exposure is associated with skin carcinogenesis. Keratinocytes are believed to be the main target cells in arsenic carcinogenesis because of stimulatory effects, such as cell activation and proliferation.\textsuperscript{49} Arsenic induces time- and concentration-dependent cellular responses leading to keratinocyte dysfunctions.\textsuperscript{49} In addition, pigmentation and keratosis are the specific skin diseases associated with chronic arsenic toxicity.\textsuperscript{21} P53 polymorphism has been found to be associated with increased occurrence of arsenic-induced keratosis. Various genes are involved in the regulation of arsenic metabolism, single-nucleotide polymorphisms of purine nucleoside phosphorylase, in one study, showed increased occurrence of arsenicosis.\textsuperscript{11,13}

Arsenic interferes with the activation and functions of immune system. Differential immune activation among the individuals might account for the different susceptibilities of humans for cancer development.\textsuperscript{44} In patients with arsenic-induced Bowen’s disease, there is a selective CD4 T-cell induced apoptosis through tumor necrosis factor-alpha pathway, decrease in macrophage differentiation and phagocytosis, reduced Langerhans cell numbers and dendrites, altered regulatory T-cell distribution and other immune alterations. Several lines of evidence from mouse and fish studies also confirmed the potent and multifaceted effects of arsenic in the immune system.\textsuperscript{44} The molecular bases of immunosuppression by arsenic in lymphocytes may include chromosomal and DNA abnormalities, decreased T-cell receptor activation, and the cellular status of oxidation and methylation.\textsuperscript{44}

5. **CALCIUM SIGNALLING AND CELL DEATH INDUCED BY ARSENIC TRIOXIDE**

Calcium is a universal second messenger. To survive, living cells need to maintain a tight control over intracellular calcium concentration, $[\text{Ca}^{2+}]_i$, dynamics that are modulated by calcium channels and calcium stores.\textsuperscript{4,52 – 56} $[\text{Ca}^{2+}]_i$ could be increased by $\text{Ca}^{2+}$-entry from the extracellular space; $\text{Ca}^{2+}$ release from the stores; impairment of $\text{Ca}^{2+}$ selective transport proteins which pump $\text{Ca}^{2+}$ in the extracellular space and/or in the calcium stores.\textsuperscript{57} Moreover, $[\text{Ca}^{2+}]_i$ could be decreased by a reduction of $\text{Ca}^{2+}$ entry by blocking calcium selective pores\textsuperscript{58,59} or by an enhancement of the efficiency of calcium transport proteins.

Besides its physiological function, $[\text{Ca}^{2+}]_i$-rises as well as deregulation of in local $[\text{Ca}^{2+}]_i$, distribution could lead to necrosis or apoptosis.\textsuperscript{56,60} The calcium stores represented mainly by mitochondria and endoplasmic reticulum (ER), play important roles in cellular $\text{Ca}^{2+}$ homeostasis and signalling: (a) regulating processes (e.g. motility, secretion, gene expression); (b) signalling cascades controlling proliferation, differentiation; (c) cell death in physiological processes or in injury and disease.\textsuperscript{52,53}

Many human diseases can be attributed to malfunction of apoptosis. This could result in: (1) cell accumulation when apoptosis is not triggered or (2) cell loss when apoptosis is abnormally triggered. Inefficient apoptosis results in uncontrolled cell growth and tumour formation. Therefore, proper function of the apoptotic process is critical for cancer treatments.\textsuperscript{61 – 67} Apoptotic cells can be recognised by morphological features. Two well-characterized apoptotic pathways determine caspase (aspartate-specific cysteine protease) activation and execution of apoptosis: (i) **the death receptor pathway** involves the interaction of a death receptor with its ligand and (2) **the intrinsic or mitochondrial** pathway that depends upon the participation of mitochondria (receptor-independent).\textsuperscript{56,63 – 68}

Calcium signalling could play a major role in $\text{As}_2\text{O}_3$ induced toxicity thus influencing the cell death by apoptosis or necrosis. Metallic compounds including arsenic interact with $[\text{Ca}^{2+}]_i$, homeostasis of living cells,\textsuperscript{14,15} and could affect the plasma membrane, mitochondria, or endoplasmic reticulum (ER).\textsuperscript{43,54} One of the many metallic compounds, $\text{As}_2\text{O}_3$, is used effectively to treat APL and could be useful in the treatment of other types of cancer.\textsuperscript{19,28,50 – 51,69} In a study of Zheng and colleagues have shown using gene expression microarray and applying $\text{As}_2\text{O}_3$ in APL NB4 cells that several molecular signalling pathways, including the activation of calcium signalling (with ER stress and involvement of calcium receptors), modulated the cell response to $\text{As}_2\text{O}_3$, with end point leading to cell death.\textsuperscript{70} It was also shown that an $\text{As}_2\text{O}_3$-triggered increase of $[\text{Ca}^{2+}]_i$ inhibited cell growth and induces apoptosis in
human malignant cell lines upon an increase of cellular H2O2, a decreased mitochondrial membrane potential and activation of caspase-3.\textsuperscript{25,69,71,72}

While it is documented that [Ca\textsuperscript{2+}]i overloads could trigger apoptosis,\textsuperscript{54} detailed work describing how As\textsubscript{2}O\textsubscript{3} induced [Ca\textsuperscript{2+}]i modulations are involved in cell death of neuroblastoma and embryonic kidney cells is presented in the studies of Florea et al.\textsuperscript{55,56} Here, it was demonstrated that As\textsubscript{2}O\textsubscript{3} triggers an irreversible increase of [Ca\textsuperscript{2+}]i, but also calcium transients. The As\textsubscript{2}O\textsubscript{3} effects on calcium homeostasis were similar in the two cell lines. Furthermore, the Ca\textsuperscript{2+}-release from intracellular calcium stores was most the important As\textsubscript{2}O\textsubscript{3}-induced effect since extracellular calcium did not significantly influence [Ca\textsuperscript{2+}]i elevation. As\textsubscript{2}O\textsubscript{3} shows minimal effect on Ca\textsuperscript{2+} entry from the extracellular space. However, As\textsubscript{2}O\textsubscript{3} is not totally excluded since it was not possible to clamp the Ca\textsuperscript{2+}-concentration in the extracellular solution because the cells did not survive the course of the experiments.\textsuperscript{55,56}

Also, it was demonstrated that IP\textsubscript{3}- and Ry-receptors are involved in regulation of As\textsubscript{2}O\textsubscript{3}-induced [Ca\textsuperscript{2+}]i rise.\textsuperscript{55,56} This could be an important finding for improving anti-cancer treatments since the involvement of ER, IP\textsubscript{3} and RyR was also proven in the work of Zheng et al.\textsuperscript{70} These authors show that calcium signalling plays an important role in As\textsubscript{2}O\textsubscript{3}-induced apoptosis of APL cells. Additionally, Zheng and coworkers have shown that calcium binding proteins (S100 family of proteins) could be also involved in calcium signalling by As\textsubscript{2}O\textsubscript{3} as well as PKC, PKA, aquaporin 9.\textsuperscript{70,73,74}

Although toxicologists have traditionally associated cell death with necrosis, a body of evidence suggests that different types of environmental and clinical relevant chemicals exert their toxicity throughout apoptosis. The pro-apoptotic mechanisms of a chemical are often unknown,\textsuperscript{64} but first hints might be represented by the mitochondria in the form cytochrome c release and other pro-apoptotic proteins, followed by caspase activation and apoptosis. Particularly in particular, the Bcl-2 family (Bax and Bak) of proteins might be involved in the release of cytochrome c, or by Ca\textsuperscript{2+}-triggered mitochondrial permeability transition; while other proteins can modulate the caspase activation (for review see\textsuperscript{70}).

As\textsubscript{2}O\textsubscript{3} is used to induce remission in patients with APL based on its tumour-specific mechanism of apoptotic induction.\textsuperscript{19,23,25,28,54,56–78} Previous studies have demonstrated that arsenic compounds cause direct damage to mitochondria.\textsuperscript{79,80} At low concentrations, arsenic stimulated cytochrome c release and apoptosis via a Bax/Bak-dependent mechanism whereas at higher concentrations (125 \textmu M-1 mM), cells died via a Bax/Bak-independent mechanism mediated by oxidative stress resulting in necrosis.\textsuperscript{79,80}

Arsenic directly inhibits the complex I of the mitochondrial electron transport chain leading to mitochondrial permeability transition (MPT), generation of ROS and thiol oxidation. These effects occurred at As\textsubscript{2}O\textsubscript{3} concentrations of 50 \textmu M and higher, in which the oxidative stress associated with these effects blocked the caspase activation. At high concentrations of arsenic, the cytochrome c release occurs indirectly via the activation of Bax/Bak rather than via direct mitochondrial damage.\textsuperscript{79,80} Furthermore, these results implicate ROS in a concentration-dependent mechanistic switch between apoptosis and necrosis.\textsuperscript{79,80}

It was affirmed that arsenic compounds are effective in the treatment of APL down regulating the Bcl-2 expression. It induces apoptosis upon releasing an apoptosis-inducing factor (AIF) from the mitochondrial intermembrane space from where it translocates to the cell nucleus.\textsuperscript{81} AIF then continues the apoptosis process, resulting in altered nuclear biochemistry, chromatin condensation, DNA fragmentation, and cell death.\textsuperscript{81} These effects result in complete remission with minimal toxicity in patients with refractory APL.\textsuperscript{82–84,87,85}

As\textsubscript{2}O\textsubscript{3} exerts a dose-dependent dual effect \textit{in vitro}; it triggers apoptosis at relatively high concentrations (0.5 to 2.0 \textmu M) associated with the collapse of mitochondrial transmembrane potentials and it induces partial differentiation at low concentrations (0.1 to 0.5 \textmu M) where the retinoic acid signalling is required for APL cell differentiation.\textsuperscript{86} As\textsubscript{2}O\textsubscript{3} down-regulated telomerase activities as well as induced about 40%- 60% of apoptosis in leukaemia NB4, K562, and HL-60 cells at the concentration of 0.6, 2.7, and 8.1 \textmu M respectively.\textsuperscript{83}

Several authors describe the cytotoxicity of As\textsubscript{2}O\textsubscript{3}; e.g. As\textsubscript{2}O\textsubscript{3} induced cytotoxicity \textit{in vitro} (human chronic leukaemia cell line) with an IC\textsubscript{50} of 10 \textmu M after 24 h of exposure.\textsuperscript{20,70} The same authors described DNA fragments, morphological changes, and chromatin condensation of the cells undergoing apoptosis after incubation with a twice higher As\textsubscript{2}O\textsubscript{3} concentration with caspase 3 and p38 activation that was confirmed by other authors using different cell lines.\textsuperscript{20,86,25} showed that 1 \textmu M As\textsubscript{2}O\textsubscript{3} had cytotoxic effects in malignant cells but not in none malignant human embryonic cells, which is
contradiction with the results of Florea et al.\textsuperscript{56} This could be explained by the use of different \textit{in vitro} models that could have different sensibility, since kidneys represent a target for As\textsubscript{2}O\textsubscript{3} toxicity. In the study of Florea et al.\textsuperscript{56} it is shown that As\textsubscript{2}O\textsubscript{3} (1 \mu M) exhibits cytotoxic effects significantly decreasing cell viability, enhanced cell death by apoptosis, and induces DNA damage in neuroblastoma as well as in HEK cells; while no As\textsubscript{2}O\textsubscript{3} application did not have an effect on calcium homeostasis and cytotoxicity.\textsuperscript{56} The increase in micronucleus and apoptotic rate could be a result of mitochondrial and ER stresses with formation of ROS, that can further damage DNA and can signal the induction of programmed cell death.\textsuperscript{56}

Additionally, As\textsubscript{2}O\textsubscript{3} concentrations of 0.01-1 \mu M applied to human malignant cell lines, MGC-803, HIC, MCF-7, HeLa, BEL-7402 and A549 cells showed growth inhibition and apoptosis in a time dose-dependent manner.\textsuperscript{25} Changes in [Ca\textsuperscript{2+}]\textsubscript{i}, correlated with the sensitivity of these cells to As\textsubscript{2}O\textsubscript{3} indicate that a critical intracellular Ca\textsuperscript{2+} signal transduction pathway could be involved in As\textsubscript{2}O\textsubscript{3} -mediated cell-death.\textsuperscript{25} Strikingly, As\textsubscript{2}O\textsubscript{3} concentrations lower than 1 \mu M were able to damage the DNA in neuroblastoma and HEK cells, however apoptosis was triggered in neuroblastoma cells but not in HEK cells, showing As\textsubscript{2}O\textsubscript{3} specificity for tumour cells.\textsuperscript{56} As shown with low As\textsubscript{2}O\textsubscript{3} concentrations, the induction of DNA damage could be a problem when apoptosis is not longer triggered because cells undergo division and proliferate with a damaged DNA. This effect could be a supplementary risk in the case of non-tumour cells, since clastogenesis increases the possibility of secondary malignancies. Cancer cell line specific effects exhibited by As\textsubscript{2}O\textsubscript{3} have been shown also by other studies.\textsuperscript{87} In human glioblastoma cell lines, As\textsubscript{2}O\textsubscript{3} induced apoptosis in A172 cells but not in T98G cells even when As\textsubscript{2}O\textsubscript{3}-induced ROS production was observed in both cell lines.\textsuperscript{85} But, it was affirmed that mitochondrial aggregation played an important role in regulating the sensitivity to As\textsubscript{2}O\textsubscript{3}-induced apoptosis.\textsuperscript{87}

Furthermore, As\textsubscript{2}O\textsubscript{3} could be effectively used for treatment of other forms of cancer. Positive effects have been documented for neuroblastoma as well as prostate, ovary and cervix carcinoma.\textsuperscript{22,82,88–90} Like APL cells, neuroblastoma (NB) cells are arrested at an early stage of differentiation; cells of highly malignant tumours fail to undergo spontaneous maturation if treated with As\textsubscript{2}O\textsubscript{3}.\textsuperscript{82} An As\textsubscript{2}O\textsubscript{3} concentration of 1 \mu M can reduce the number of viable NB cells after 72 h of exposure \textit{in vitro}. The IC\textsubscript{50} in six different neuroblastoma cell lines treated for 3 days was between 1.5 to 5 \mu M, the most sensitive being SK-N-BE(2) cells (derived from a chemotherapy resistant tumour). As\textsubscript{2}O\textsubscript{3} induced apoptotic death of NB cells and involved decreased expression of Bcl-2 and stimulation of caspase-3 activity.\textsuperscript{82} The effect of As\textsubscript{2}O\textsubscript{3} was also investigated \textit{in vivo}, in nude mice bearing tumours of NB cells. As\textsubscript{2}O\textsubscript{3} treatment reduced tumour growth but complete remission was not achieved. Therefore, it was suggested that As\textsubscript{2}O\textsubscript{3} in combination with existing treatment modalities, might be a treatment approach for high-risk neuroblastoma patients.\textsuperscript{82} Nevertheless, the effects of As\textsubscript{2}O\textsubscript{3} combinations involve several molecular networks, including transcription factors and cofactors, activation of calcium signalling (especially endoplasmic reticulum related calcium events), stimulation of the interferon pathway, activation of the proteasome system, restoration of the nuclear body, cell-cycle arrest, and gain of apoptotic potential.\textsuperscript{70}

Arsenic compounds can inhibit growth and induce apoptosis in human ovarian and cervical cancer cells (C180 – 13S, OVCAR and HeLa cells) at clinical relevant concentrations indicating that these compounds could be effectively used for treating gynecological cancer.\textsuperscript{89,91} In addition, As\textsubscript{2}O\textsubscript{3} could sensitize human cervical cancer cells to ionizing radiation \textit{in vitro} and \textit{in vivo}.\textsuperscript{89} This has a synergistic effect in decreasing clonogenic survival and in the regression of established human cervical tumours.\textsuperscript{89} Apoptosis by a combined treatment of As\textsubscript{2}O\textsubscript{3} and radiation was associated with ROS generation and loss of mitochondrial membrane potential, resulting in the activation of caspase-9 and caspase-3, increased G2/M cell cycle distribution at the concentration of As\textsubscript{2}O\textsubscript{3} which did not alter cell cycle when applied alone.\textsuperscript{89} \textit{In vivo}, As\textsubscript{2}O\textsubscript{3} induces a high complete remission rate in patients with both primary and relapsed APL (85% – 90%).\textsuperscript{22,85} After complete remission obtained in relapsed patients, chemotherapy in combination with As\textsubscript{2}O\textsubscript{3} as post-remission therapy has yielded better survival than treatment with As\textsubscript{2}O\textsubscript{3} alone.\textsuperscript{84,22,85}

**OUTLOOK**

Nearly 100 million people are at risk because of arsenic contaminated drinking water.\textsuperscript{6,92} WHO guideline recommends that levels of arsenic in drinking water should not exceed 10 \mu g/L, but more than 30 million people drink arsenic-containing water with higher arsenic levels (>50 \mu g/L) especially...
in Bangladesh and India.\textsuperscript{94} Thus, it was recognized that the impact of high arsenic exposure on human health is much more complicated than originally anticipated as it resulted in various diseases including cancers. The pathology behind arsenic exposure that leads to diseases remains still unclear.\textsuperscript{92} Furthermore, disease outcome is likely dependent on cell-type-specific responses and interaction with individual genetics, other toxicants, and infectious agents influencing: gene expression, epigenetic profiles, and tissue biomarkers.\textsuperscript{6,114}

Despite its many therapeutic qualities, $\text{As}_2\text{O}_3$ has been mentioned mainly as a poison and public health problem than as an effective anticancer drug. The ability of $\text{As}_2\text{O}_3$ to treat APL has changed the point of view in regard with its positive evaluation.\textsuperscript{32} Arsenic affects many cellular and physiological pathways, and therefore malignancies such as hematologic cancer as well as solid tumors might be treated with $\text{As}_2\text{O}_3$.\textsuperscript{74} Research is focussed on arsenic compounds bringing new insights into the pathogenesis of tumors and a rising hope that arsenic compounds might be useful in treating other types of cancer. Thus, these multiple actions of $\text{As}_2\text{O}_3$ also show the need for additional mechanistic studies to determine which actions mediate the diverse biological effects of this agent. This information will be critical to realize the potential for synergy between $\text{As}_2\text{O}_3$ and other chemotherapeutic agents, thus providing enhanced benefit in cancer therapy.\textsuperscript{32,72}

REFERENCES


Robertson JD, Orenius S. Role of mitochondria in toxic cell death. Toxicology. 2002;181:491–496.


