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# SOME ASPECTS OF ARSENIC TOXICITY AND CARCINOGENICITY IN LIVING ORGANISM WITH SPECIAL REGARD TO ITS INFLUENCE ON CARDIOVASCULAR SYSTEM, BLOOD AND BONE MARROW

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**Abstract.** This paper gathers data on the most current aspects of arsenic action, especially its influence on the cardiovascular system, blood and bone marrow. A potential carcinogenic mechanism of arsenic is also discussed.
Arsenic is a potent toxicant that may exist in several valencies and in a number of inorganic and organic forms. Most cases of arsenic-induced toxicity in humans are due to exposure to inorganic arsenic, and there is an extensive database on the human health effects of common arsenic oxides and oxyacids.
Exposure of humans living near hazardous waste sites may involve inhalation of arsenic dusts in the air, ingestion of arsenic in water, food or soil, or dermal contact with contaminated soil or water.
The exposure to arsenic via the inhalation route is responsible for the increased risk of lung cancer, although respiratory irritation, nausea and skin effects may also occur.
The oral route of exposure to arsenic predominates in the general population. The most common effects of arsenic ingestion are gastrointestinal irritation, peripheral neuropathy, vascular lesions, anemia, skin diseases, including skin cancer and other cancers of the internal organs like bladder, kidney, liver or lung.
Relatively little information is available on the effects of direct dermal contact with inorganic arsenicals, but several studies indicate local irritation and dermatitis as the major ones.

**Key words:**
**Arsenic, Toxicity, Carcinogenicity, Cardiovascular system, Blood, Bone marrow**

## ARSENIC POISONING

**Arsenic (As)** belongs to the fifth group of Mendeleyev's periodic table. As a free element arsenic appears in two allotropic forms: grey and yellow (cristalline) [1]. Environmentally it can be found as a sulphuric compound in the lead, copper, nickel and ferrous ore, and, in small quantities, in the soil. The main way of arsenic natural circulation is water. In Russia, New Zealand, Romania and the USA, drinking water is also arsenic-contaminated and it causes serious intoxications among people.

In the air, arsenic takes the form of inorganic compounds (e.g. arsenic trioxide). Volcano's eruption and purposeful activities of people are the main reasons for releasing this toxin to the atmosphere.

Inorganic arsenic compounds enter the human body via the lung and gastrointestinal tract.

**Acute arsenic poisoning** is uncommon. It is usually the result of accidental or suicidal ingestion of insecticides or pesticides [2–6]. Toxicity is manifested by the stomach and intestine damage (diarrhea, vomiting, hemorrhage, elec-

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trolyte disturbances), muscle function disorders (fasciculation, myoclonia), cardiac arrhythmia and face oedema. Arsenic acts as a local irritant causing local skin inflammation, ulceration and perforation of the nasal septum. Some arsenicals may also function as contact allergens. Hematological abnormalities include anemia, leukopenia and thrombocytopenia, secondary to depression of bone marrow [7, 8]. Megaloblastic erythropoiesis is unusual and has been reported occasionally. In such a case megaloblastosis is thought to be a direct toxic effect of arsenic (inhibition of nucleic acid synthesis), not $B_{12}$ and folate deficiency [9]. Leukopenia is a prominent finding and, in fact, the major change is an absolute neutropenia in which the count is less than 1000. The number of lymphocytes is also decreased, but the decrease is not so striking as in neutropenia. The peripheral blood smear shows varied degrees of anisopoikilocytosis, and in the bone-marrow examinations a partial maturation arrest of myelopoietic elements can be observed. Rezuke et al. [10] presented a myelodysplastic syndrome as a result of arsenic intoxication. A 41-year-old woman with gastrointestinal and neurological symptoms was found to be pancytopenic. A bone marrow aspirate revealed dysmyelopoietic changes involving all three marrow cell lines.

Symptoms of **subacute and chronic arsenic intoxication** concern generally the respiratory, alimentary, cardiovascular and nervous systems or bone marrow [11–14]. Many pathologies involve the peripheral vascular system; its damage is demonstrated as Raynaud's phenomenon, cyanosis, microcirculation impairment, and finally the tissue necrosis. The most important organ involved by arsenic is the liver. The toxic effect is not restricted only to the disorder of hepatocytes function and the increased level of enzymes; it is often observed that the liver fibrosis leads to chronic hepatic failure.

## ARSENIC TOXICITY

The results of epidemiological investigations show that **arsenic** contained in **drinking water** impairs the skin. Skin lesions include hyperkeratosis, papillas and corns of hands and feet, hyper- and hypopigmentary areas of the face, neck or back [12,14]. People exposed to arsenic often present lesions of the central nervous system, including polyneuropathy, starting with numbness and tingling sensations of hands and feet, followed by paresis and hyperpathy [11–13].

The **mechanism of arsenic toxicity** consists in its transformation in metaarsenite, which acylates protein sulfhydryl groups, but is not bound to DNA [1,15,16]. A particular target in the cell is the mitochondria, which accumulates arsenic. Just in the mitochondria arsenic inhibits succinic dehydrogenase activity and can uncouple oxidative phosphorylation. The resulting fall in ATP levels affects all cellular functions, such a Na/K balance or protein synthesis.

The effect of arsenical compounds on GSH-related enzymes (important antioxidants) *in vitro* was also investigated. The results do not implicate a direct interaction of As with glutathione-reductase, peroxidase and transferase in the mechanism of arsenic toxicity, although As (III) appears to be an effective inhibitor of all of them [17,18].

Inorganic arsenicals change the expression of genes related to stress, which produce some proteins or activate enzymes, including: heme oxygenase, heat shock protein-60, -70 and –90, DNA damage inducible protein GADD 45 and DNA excision repair protein ERCC1. Downregulation of certain cytochrome P450 enzymes and activation of the c-Jun/AP-1 transcription complex occurred with arsenic treatments. Increases in caspase-1, TNF-alpha and the metal-responsive transcription factor MFT-1 is also evident. All these events are responsible for arsenic cytotoxicity in some types of cells, for example in the human liver cancer cells ($HepG_2$). This effect is potentiated by atrazine [19–21].

Trivalent arsenicals are potent inhibitors of thioredoxin reductase (NADPH-dependent flavoenzyme), so they inhibit the cellular response to oxidative stress [22].

Considerations of the biochemical basis of heavy metal detoxification in animals have focused exclusively on two classes of peptides, thiol tripeptide, glutathione (gamma-Glu-Cys-Gly), and a diverse family of cysteine-rich low molecular weight proteins, the metallothioneins. Plants and some fungi, however, not only deploy GSH and metallothioneins for metal detoxification but also synthesize

another class of heavy metal binding peptides termed phytochelatins (PC) from GSH. PC-mediated heavy metal detoxification is not restricted to plants and some fungi, but extends to animals. The ce-pcs-1 gene of the nematode worm *Caenorhabditis elegans* encodes a functional PC synthase whose activity is critical for heavy metal tolerance in the intact organism [16,18,23].

Inorganic arsenicals (arsenite and arsenate) are strongly **toxic to macrophages** [24,25]. These forms mainly cause necrotic cell death with partially apoptotic cell death. The inorganic arsenicals also induce marked release of an inflammatory cytokine – TNF alpha – at cytotoxic doses. This strong cytotoxicity might be mediated via active oxygen and protease activation.

The mechanism of arsenic-induced cross-tolerance to cytotoxicity, genotoxicity and apoptosis induced by other metals (nickel for instance) appears related to a generalized resistance to oxidant-induced injury, probably based at the increased cellular GSH level [26].

Viability testing showed that relative toxicities of arsenicals are as follows: As (III) > Mas (III) > DMAs (III) > DMAs (V) > Mas (V) > As (V). Trivalent arsenicals increase cell proliferation at low concentrations (0.001–0.01 $\mu$mol). Pentavalent arsenicals do not stimulate cell proliferation. Exposure to low doses of trivalent arsenicals stimulates secretion of the growth-promoting cytokines: GM-CSF, TNF-alpha. DMAs (V) reduces cytokine production at concentrations at which proliferation is not affected. These data suggest that methylated arsenicals can significantly affect viability and proliferation of human keratinocytes and modify their secretion of inflammatory and growth-factor cytokines [27].

## CARDIOVASCULAR ABNORMALITIES CAUSED BY ARSENIC

As mentioned earlier – arsenic is a naturally occurring element that is ubiquitous in the environment. Chronic consumption of arsenic-contaminated water is epidemiologically linked to many toxic effects including hyperpigmentation, keratosis, peripheral neuropathy, skin and lung cancer and peripheral vascular disease. The full manifestation of arsenic peripheral vascular disease is referred to as **"blackfoot disease"** (BFD) [28–34]. Clinically, blackfoot disease is characterised by numbness or coldness in the extremities followed by various symptoms that may include acrocyanosis, secondary Raynaud's phenomenon, claudication, ulceration and gangrene of the extremities. Gangrene is accompanied by arteriosclerosis and thromboangiitis obliterans.

Little is known about the mechanism of vascular arsenic damage, although it does appear to affect small arterioles. Perhaps non-lethal doses of arsenic increase intracellular oxidant levels, promote nuclear translocation of trans-acting factors, and are mitogenic [35,36]. The activated oxidant-sensitive endothelial cell signaling may lead to vascular dysfunction. The critical initial step in that signaling can be an activation of the plasma membrane NADPH oxidase complex as a primary source of the reactive oxygen stimulated by arsenite [37]. The superoxide radical and hydrogen peroxide ($H_2O_2$) are the predominant reactive species produced by endothelial cells after arsenite exposure [36].

Yu et al. [38] examined the factors related to endothelial cells (EC) damage in blackfoot disease. They investigated the effects of purified IgG collected from BFD patients and found that: (1) EC binding activity of BFD-IgG was significantly higher than that of normal IgG. (2) BFD-IgG at high concentrations induced EC cytotoxicity. (3) BFD-IgG at a concentration of 100 $\mu$g/ml stimulated neither the release of von Willebrand factor nor the expression of intracellular adhesion molecule-1 by EC. The authors suggest that only persons who produce the IgG anti-endothelial cell antibody are potential BFD victims.

The present data indicate that arsenite initiates endothelium dysfunction, at least partly, by suppressing the Fas ligand expression through activating reactive oxygen species sensitive to endothelial cell signaling [39].

Investigations of Yang et al. [40] revealed that plasma protein C activity is enhanced by arsenic, but inhibited by fluorescent humic acid associated with BFD. In the presence of humic acid, the enhancement effect of arsenic oxide was completely abolished, resulting in concentration-dependent inhibition of protein C activity. Since protein C is a potent anticoagulant and profibrinolytic agent,

acquired defects of protein C induced by humic acid might cause a thrombophilic or hypercoagulable state. This may be one of the possible mechanisms of humic acid-induced thrombotic disorders in BFD.

There is also found that organometallic complexes composed of humic acid (HA) and arsenic show enhanced inhibition of plasmin activity as compared with either humic acid or arsenic alone [41]. Oxidative stress may play a role in that inhibition, such as ascorbic acid, alpha-tocopherol, catalase and superoxide dismutase abrogate the inhibitory effects of HA and HA-arsenic complexes. Both of these agents are etiological factors in the development of peripheral vascular diseases, like BFD.

At present it is known that As (III) inhibits the growth of cultured human umbilical vein endothelial cells and diminishes the expression of agglutinin I lectin, a marker for vascular endothelium. GSH treatment antagonized the cytotoxicity of As III. The cytoprotective effect of GSH is attributed to the induction of prostacyclin synthesis [28].

Peripheral vascular disease has been reported in individuals exposed to As in occupational settings. This effect can be severe and long-lasting. A high prevalence of endangiitis obliterans and acrodermatitis atrophicans was observed in workers whose exposure to As had ended over 30 years earlier [29,33,34,42]. The cumulative life-time incidence of BFD is estimated to be less than 10% among individuals who consume As-contaminated drinking water. Certain occupations (fishermen, salt-field workers), low socio-economic status and undernutrition are factors of increased risk.

Occupational and environmental exposure to inorganic arsenic is associated with the occurence of Raynaud's phenomenon and objectively registered abnormal finger systolic blood pressure at local cooling [30,31]. A subnormal finger systolic blood pressure indicates a vasospastic tendency (Raynaud's disease). Because these changes are not reversed over a long period and seem to be unrelated to the most recent urinary arsenic excretion, altered regulation of peripheral vascular tone may be an irreversible consequence of long-term exposure to arsenic.

Yu et al. [34] described the progress of vascular lesions in BFD from an early erythematous stage through an intermediate ulcerative stage to a later chronic arsenism stage. Morphologically the erythematous stage was characterised by fibroid degeneration of blood vessel walls and a perivascular small-cell infiltration. Thrombotic occlusion and proliferation of the *vasa vasorum* occurred during the ulcerative stage. In the chronic arsenism stage the lumens of blood vessels were completely occluded and the affected extremity was gangrenous [29].

Based on **concentrations of some elements in urine** – the investigators evaluated vascular changes in blackfoot disease [43]. The copper concentration in urine changed slightly for all clinical stages, whereas the concentrations in blood and hair showed less correlation with BFD stages. Zinc concentration was highest in the fourth stage; zinc appeared to be associated with the occurrence of scaling and cracking of the skin, and even with feet ulceration and gangrene. Arsenic, which is claimed to be a major causative agent of BFD, increased from the zero stage and showed a particularly high concentration in the second stage. The maintained increase in As level in the blood and urine considerably complicated peripheral vasculopathy. The selenium concentration decreased from the zero stage, showing its lowest value during the second stage, then increased in the later stages. The decrease in selenium was attributed to the antagonistic effect of arsenic; selenium was retained during the initial stages.

There is evidence that arsenic affects the structure and function of the cardiovascular system and seems to be a reason for the altered myocardial depolarization (prolonged Q-T interval, nonspecific S-T segment changes), arrhythmias or hypertrophy of the ventricular wall [44–48]. Taylor [32] found significant increase in the standardized mortality ratio (SMR) for tuberculosis, malignant neoplasms, liver cirrhosis and heart diseases. The SMR for cerebrovascular disease did not increase. Arsenic trioxide, which is used for the treatment of acute promyelocytic leukemia, is responsible for the prolongation of QT interval and ventricular tachycardia [47].

Several environmental toxins: lead, asbestos, arsenic, cadmium and others produce both hypertension and cardiac arrhythmias and they are probably related to primary lung disease and secondary to heart disease [32]. Possible

mechanisms that may induce cardiovascular diseases include:

- damage to the endothelial barrier in the vascular system;
- activation of leukocytes and platelets;
- initiation of plaque formation;
- stimulation of the inflammatory response;
- kidney-related hypertension;
- direct damage to cardiac and blood vessel tissues.

Recently, a team of epidemiologists have studied biological markers for cardiovascular diseases in a group of 40 workers occupationally exposed to arsenic. The concentration of glycosylated hemoglobin (Hgb $A_1C$) was increased in whole blood of the As-exposed workers. They conclude that arsenic exposure **influences carbohydrate metabolism**, increases the systolic blood pressure, and finally may result in the increased risk of cardiovascular diseases [49].

It was also suggested that arsenite's **atherosclerosis** may occur due to activated NADH oxidase to produce superoxide, which then causes oxidative DNA damage in vascular smooth muscle cell [36,48,50,51].

## ARSENIC AND HEPATIC VASCULATURE

Arsenic exposure can affect **hepatic vasculature** and induce noncirrhotic portal hypertension [52]. Here, enhanced collagen synthesis in intrahepatic portal venules obliterates the portal circulation, resulting in the development of collateral vessels and hypoplastic changes in the portal tract [53]. The mechanism of arsenic affection of the portal vasculature is unknown, therefore the involvement of the endothelial cells of the vasculature in response to As exposure has become a topic of interest. Labadie et al. [54] suggested that obliteration of portal venules in noncirrhotic portal hypertension was the consequence of the injury and repair of the vascular endothelial cells by As. The induction of angiosarcoma in the As-exposed workers has been attributed to the carcinogenic transformation of endothelial cells of the liver vasculature. Investigations carried out over thirty years ago [53] indicated that noncirrhotic portal hypertension was an effect of portal tract fibrosis and the increase in the number of portal veins. Histological examinations showed slight sclerosis around the portal veins and the hypertrophic muscular walls inside the vessel.

Hepatic fibrosis due to long-term arsenic toxicity is associated with the hepatic membrane damage, probably caused by reduction of glutathione and antioxidant enzymes (glucose-6-phosphate dehydrogenase, catalase, GSH-peroxidase, GSH-reductase and GSH-S-transferase). The activity of plasma membrane Na/K ATPase is also reduced [52,55].

## MECHANISMS OF THE ARSENIC ANEMIA

While arsenic toxicity to the blood vessels is already established, **toxicity to erythrocytes** has not as yet been evaluated in epidemiological studies. Toxicity to erythrocytes can be manifested as a change in the shape or deformability of the cell [56–61]. Abnormalities in red cell deformability can lead to disturbances in microcirculation and contribute to ischemia and tissue damage. An example of this is sickle-cell anemia.

The morphologically changed erythrocytes in scanning electron microscopy were classified according to Bessis as:

- normal or discocyte, smooth flat red blood cell with biconcave shape and <3 nodules on the surface;
- echinocyte II with >3 protuberances (spicules);
- echinocyte III with multiple spicules;
- spheroechinocyte [59].

The morphologic transformation of erythrocytes results from the decreased level of intracelullar ATP, induced by arsenic. This leads to the structure changes of the erythrocyte membrane and the differences in the osmotic resistance of erythrocytes. On the other hand, arsenic as the phosphorane analogue, provokes arsenolysis of mitochondrial enzymes.

The major effect of exposure to **arsine ($AsH_3$)** is anemia due to massive **intravascular hemolysis**. The earliest indicators of erythrocyte damage are changes in sodium and potassium levels [58]. The cells lose volume control, manifested by leakage of potassium, influx of sodium and increase in hematocrit. Arsine does not alter ATP levels

nor inhibit ATPases. These changes are followed by disturbances in membrane ultrastructure. Such events produce hemolysis, which appears to be dependent on membrane disruption [61].

Although $AsH_3$ is a reducing agent, it has been postulated that it damages cells through oxidative mechanisms, possibly through the formation of hydrogen peroxide ($H_2O_2$) and inhibition of catalase. Moreover, this oxidative stress can be reversible following combined administration of N-acetylcysteine and meso 2,3-dimercaptosuccinic acid [62].

Reactive oxygen species can be produced in biological systems through the action of exogenous compounds or during normal cellular functions. Red blood cells contain an extensive antioxidant system of enzymes and co-factors. However, when this system is overwhelmed, hemoglobin-$O_2$ ($HbO_2$) contained in red blood cells (RBC) is a vulnerable target for reactive oxygen species. The result of this attack on hemoglobin-$O_2$ is oxidation of the heme iron, followed by alterations in the structure of heme component, globin and heme-globin interactions. The resultant destabilization of the molecule induces precipitation of protein (Heinz bodies). The effects of Heinz bodies include redistribution of membrane proteins and increased membrane rigidity, while the effects of hemin include oxidation of membrane protein sulfhydryl groups, dissociation of membrane skeletal proteins and perturbation of membrane ion gradients.

**The mechanisms of $AsH_3$ and $H_2O_2$ actions are different.** $AsH_3$ causes hemichrome and methemoglobin formation, while $H_2O_2$ treatment results in the formation of ferrylhemoglobin. The hemoglobin damage depends on catalase activity. High-activity catalase has little effect on the initial hemoglobin-$O_2$ changes caused by $AsH_3$. The similar effect can be observed in glutathion-peroxydase. In summary, the investigators have found little evidence to suggest the involvement of $H_2O_2$, superoxide anion and hydroxyl radical in the hemoglobin destruction (except for the late effect of membrane protein precipitation). The following equation demonstrate possible reactions involving $AsH_3$ and $HbO_2$:

$$H_2\text{-As-H} + HbO_2 = H_2As + HbO_2\text{-H} = \text{met-Hb} + H_2\text{As-OOH}$$

Alternatively, the reaction may produce hydrogen peroxide and an arsenic adduct, such as arsine-hemoglobin ($H_2$As-Hb) or arsine-heme ($H_2$As-heme).

Arsine-hemoglobin adduct or arsine-heme adduct has not as yet been identified [56,57].

Low doses of arsenic disrupt normal structure and function of platelets [8]. After arsenic treatment the cell margins appears irregular and wavy with small pseudopodia-like protrusions from the surface. In this case arsenic induces the platelets' aggregation through the cytosolic cAMP, which is decreased by the metal–induced inhibition of phosphodiesterase.

## AN AFFECTION OF THE IMMUNOLOGICAL SYSTEM

There is also evidence that arsenic and other metal toxicants **affect the immunological system**. Broeckaert et al. [63] investigated the effect of intratracheally instilled coal flay ash and copper smelter dust on the lung integrity and on the release of tumor necrosis factor alpha (TNF-alpha) by alveolar phagocytes. They hypothesized that suppression of TNF-alpha production is dependent upon the slow elimination of the particles and their metal content from the lung.

Exposure to a single intratracheal administration of gallium arsenide (GaAs) has been shown to suppress antibody production, as well as other T cell-mediated immunological functions [64]. GaAs has also been shown to exert toxic effects on events occurring early in the antibody-forming cell response, which may include lymphocyte activation and proliferation. The isolated T cells exposed to GaAs were significantly suppressed in their ability to proliferate to concavalin A, phytohemagglutinin and anti-CD3 epsilon plus interleukin-2. In addition, the expression of CD25, leukocyte function antigen-1 and intercellular adhesion molecule-1 were significantly below normal.

According to some authors, phagocyte-mediated oxidant damage to vascular endothelium is likely to be involved in various vasculopathies caused by arsenic, including pulmonary leak syndromes, such as adult respiratory distress syndrome. Balla et al. [65] have shown that heme,

a hydrophobic iron chelate, is rapidly incorporated into endothelial cells, where it markedly aggravates cytotoxicity engendered by polymorphonuclear leukocyte oxidants or hydrogen peroxide. This protection is associated with the induction of mRNA for both heme oxygenase and ferritin. Ferritin inhibits oxidant-mediated cytolysis and, by this way, counteracts the damage of pulmonary vascular barrier.

Trivalent arsenic compounds can inhibit enzymatic activity of the lysosomal protease cathepsin L (CathL) in the murine antigen-presenting B cell line TA3. CathL plays an important role in antigen processing, the mechanism by which antigen-presenting cells cleaves foreign protein antigens to peptides for stimulating a T cell response. Deficient proteolysis may lead to the diminished immune responses [66].

## ASPECTS OF ARSENIC CARCINOGENICITY

The second – near by toxic – important effect of arsenic is its **probable mutagenic activity** and **proved carcinogenicity** caused by clastogenesis in peripheral lymphocytes and sister chromatid exchange [67–70]. Arsenic does not act through classic genotoxic and mutagenic mechanism, but rather at the level of tumor promotion by modulating the signaling pathways responsible for cell growth. The As carcinogenic activity results generally from the inhalation exposure [71]. The cancer develops first of all in the lung and skin (ca basocelullare, squamous cell carcinoma), although it can involve the bladder, liver and kidney.

Activating protein-1 (AP-1) is a functionally pleomorphic transcription factor regulating diverse gene activities. Increased AP-1 binding activities caused by activation of mitogen-activated protein kinases (MAP-kinases) pathway could be the one of precursor markers in arsenic-induced cancers. Marked AP-1 DNA-binding activity only occurred at exposure levels of sodium arsenite above 20 microg/ml [72,73].

Dimethylarsinic acid (DMA) has been used as a herbicide (cacodylic acid) and is the major metabolite formed after exposure to tri- (arsenite) or pentavalent (arsenate) inorganic arsenic via ingestion or inhalation.

Kashiwada et al. [74] investigated the cytogenic effects of DMA on bone marrow cells. DMA increased mitotic indices significantly 16, 24, 48 h after administration. The similar (although stronger) effect was observed after colchicine treatment. DMA significantly induced aneuploidy, which might be associated with carcinogenicity of arsenic, and induced an organ-specific lesion – single strand breaks in DNA. Mechanistic studies have suggested that this damage was mainly due to peroxyl radical and production of active oxygen species by tissues. DMA induced DNA damage also in the lung (by formation of various peroxyl radical species). There are some reports that it also could increase point mutations (G:C to T:A transversion). As appears, DMA could act as a promotor of urinary bladder, kidney, liver, lung and thyroid gland cancers [25,42,75–78].

It has also been suggested [79] that lung cancer observed among arsenic ore smelters and skin cancer among people exposed therapeutically to Fowler's solution, have as their common origin the genotoxic arsenite ion $AsO_2$-, which is active in the bone marrow micronucleus assay.

Interestingly, arsenic has also been used as an effective chemotherapy agent for certain human cancers for hundreds of years in both traditional Chinese and Western medicine [80–84]. Having considered previous findings that p53 mutations are involved in about 50% of all human cancers and that nearly all chemotherapeutic agents kill cancer cells mainly by apoptotic induction, Dong [85] suggests that arsenic may be a useful agent for the treatment of cancer with p53 mutation. These suggestions result from the observation, that low As concentrations (<25 μM) induce cell transformation (through extracellular regulated kinases – ERK phosphorylation), while higher concentrations induce cell apoptosis (activation of c-Jun NH2-terminal kinases).

Cell death induced by arsenic trioxide is associated with mitochondrial membrane depolarization, release of cytochrome c from the mitochondria, activation of caspases, poly (ADP-ribose) polymerase cleavage and internucleosomal DNA fragmentation. Moreover, the decreased glutathione content associated with the differentiation process amplifies the ability of arsenic trioxide to activate the mitochondrial pathway to cell death [86].

The treatment of promonocytic U937 cells with arsenic trioxide leads to G2/M arrest, which is associated with a dramatic increase in the levels of cyclin B and cyclin B-dependent kinase and apoptosis. Apoptosis occurs after bcl-2 phosphorylation and caspase -3 activation [87].

Wang et al. [84] observed that inorganic arsenic trioxide ($As_2O_3$) and melarsoprol were recently shown to inhibit growth and induce apoptosis in acute promyelocytic leukemia and chronic B-cell leukemia cell lines. They found that both compounds inhibited cell growth, induced apoptosis, and downregulated bcl-2 protein in all cell lines tested. The bone marrow and peripheral blood examination showed an increase in myelocyte-like cells and degenerative cells after 2–3 weeks of $As_2O_3$ treatment, and a decrease in leukemic promyelocytes. Both $As_2O_3$ and melarsoprol inhibited colony-forming unit (CFU), erythroid and CFU granulocyte-monocyte formation in cultures of PML+ and PML- progenitors. These results suggest that $As_2O_3$ and melarsoprol may be used for the treatment of leukemias. The combination of induction of apoptosis and partial differentiation could be the main cellular mechanisms of this treatment.

Some authors [80,83] found that $As_2O_3$ administered intravenously alone or in combination with chemotherapeutic drugs at a dose of 10 mg/d resulted in clinical complete remission in 90% of patients. During the treatment with $As_2O_3$ there was no bone marrow depression.

An important role in the development of leukemias is played by endothelium and angiogenesis. According to some investigators, the mechanism by which $As_2O_3$ exerts its antileukemic effect consists in apoptosis of leukemic and endothelial cells and in inhibiting leukemic cell vascular endothelial growth factor (VEGF) production [82].

Arsenic trioxide may induce clinical remission in patients suffering from APL through induction of apoptosis and activation of caspases. There was investigated the potential use of $As_2O_3$ in human gastric cancer and its possible mechanisms. Arsenic trioxide increases the activity of caspase-3, inhibits cell growth and induces apoptosis involving p53 overexpression [88].

In preliminary cell culture studies, liposomes composed of arsonolipids or phospholipid-arsonolipid mixtures, demonstrate a specific toxicity against cancer cells [89].

As a novel anticancer agent for treatment of solid cancers, $As_2O_3$ is promising, but there are no *in vivo* experimental investigations of its efficacy in the treatment of solid cancers at clinically obtained concentrations. In addition, the cell death mechanism of $As_2O_3$ has yet to be clarified, especially in solid cancers. In the study of Maeda et al. [90], human androgen-independent prostate cancer cell lines, PC-3, DU-145, and TSU-PR1 were examined as cellular models for $As_2O_3$ treatment, and $As_2O_3$-induced cell death and inhibition of cell growth and colony formation were evaluated

In all the three cell lines, arsenic trioxide at high concentrations induced apoptosis and, at low concentrations, growth inhibition. It activated p38, JNK, and caspase-3 dose dependently. This study proves that $As_2O_3$ provides a novel, safe approach to treatment of androgen-independent prostate cancer.

In spite of its incidental therapeutic properties, the International Agency for Research on Cancer (IARC) has classified arsenic as a carcinogen, for which there is sufficient epidemiological evidence to support a causal relationship between exposure and cancer. At present nine different possible modes of action of arsenic carcinogenesis are discussed: chromosomal aberrations, oxidative stress, altered DNA repair, altered DNA methylation patterns, altered growth factors secretion, enhanced cell proliferation, promotion or progression of tumor, gene amplification, and finally, suppresion of p53 gene [16,69,76,85,91].

Unlike the large majority of substances considered as human carcinogens based on the basis of epidemiological evidence, arsenic alone does not induce cancer in rodent models. That is why arsenic has sometimes been called “a paradoxical human carcinogen” [92]. Only several papers present to date *in vivo* animal studies on arsenic carcinogenicity. The investigators suggest the role of arsenic as an enhancer of ras-oncogene expression and myc activation in the development of animal skin cancer [93,94].

There is evidence that c-myc overexpression (correlated with cellular hyperproliferation, DNA hypomethylation) is mechanistically important in arsenic-induced malignant transformation. In addition, arsenite could induce in hepatic cells cytokeratin-18 expression, which is tightly correlated with differentiation programs [94].

The study by Rossman indicates that human cells lack the inducible tolerance to arsenite observed in hamster cells [68].

The genotoxicity database for arsenic indicates that it does not induce point mutations or DNA adducts, but sister chromatid exchanges and chromosomal aberrations [67,68]. Arsenic inhibits transcription of the hTERT gene, which encodes the reverse transcriptase subunit of human telomerase. The decreased telomerase activity leads to chromosomal lesions, which promote either genomic instability and carcinogenesis or cancer cell death [95].

Chromosomal aberrations were observed in genes involved in cell-cycle regulation, signal transduction, stress response, apoptosis, cytokine production, and growth factor and hormone receptor production or various oncogenes [96,97].

The mutagenicity of arsenite was assayed in a transgenic cell line, where deletions and point mutations were revealed [68]. Arsenic can also potentiate mutagenicity observed with other chemicals. This potentiation may be the result of direct interference by arsenic with DNA repair processes, perhaps by inhibiting DNA ligase [98]. Inorganic trivalent arsenicals are especially good inhibitors of enzymes containing vicinal sulfhydryl groups. Arsenic trioxide inhibits the removal of pyrimidine dimers from DNA after UV irradiation [68,91,99].

Finally, arsenic can induce DNA amplification [100].

According to the present data, molecular mechanism of arsenic carcinogenicity includes:

- hypermethylation of cytosine in the promoter region of the tumor suppresor gene p53, leading to the cell transformation [16,91,101,102];
- secretion of growth factors: granulocyte macrophage – colony stimulating factor (GM-CSF), transforming growth factor alpha (TGF-alpha) and the proinflammatory cytokine tumor necrosis factor alpha (TNF-alpha), especially in keratinocytes, leading to the epidermal neoplasia [103].

Inorganic arsenic is enzymatically methylated, consuming S-adenosyl-methionine (SAM) in this process [102]. The fact that DNA methyltransferases require the same methyl donor suggests a role for methylation in arsenic carcinogenesis. There is a hypothesis that arsenic-induced initiation results from DNA hypomethylation caused by continuous methyl depletion. Thus arsenic can act as a carcinogen by inducing DNA hypomethylation, which in turn facilitates aberrant gene expresion (p53) by its hypermethylation.

This hypothesis was also confirmed in the animal model. Tice et al. [104] evaluated the effect of hepatic methyl donor (HMD) status on the ability of sodium arsenite to induce DNA damage in HMD-deficient (HMDD) and HMD-sufficient (HMDS) mice. Choline-deficient diet prior to treatment altered arsenical metabolism. Treatment with sodium arsenite once or four times induced a significant decrease in DNA migration in the bladder and liver parenchymal cells of HMDS mice, but in skin cells of HMDD mice. Both HMDD and HMDS mice exhibited a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow. These results indicate that hepatic methyl donor deficiency significantly decreases the total urinary excretion sodium arsenite and markedly modulates target organ of arsenic-induced DNA damage.

In the early 1980s, the multistage theory of carcinogenesis and its implications for evaluating the effect of exposure to carcinogens in the workplace was described [105]. It predicts different relationships between excess carcinogenic risk and duration of exposure, age at initial exposure and follow-up time since cessation of exposure. According to this theory arsenic appears to exert a definite effect on a late stage of the carcinogenic process, like carcinogenic progressors. At the initial stage arsenic exerts an additional effect.

Later investigations indicate that arsenic could play a role as co-stimulant of mitosis similar to interleukin-1 [68].

So arsenic has been shown to be genotoxic in a wide variety of different experimental set-ups and biological end-

points. *In vitro* arsenic was shown to induce chromosomal mutagenicity like micronuclei, chromosomal aberrations and sister chromatid exchanges. It mainly acts as clastogen but also has an aneugenic potential. Its potential to induce point mutations is very low in bacterial, as well as in mammalian cell systems [106]. The available data indicate that arsenic may act indirectly on DNA, i.e. via mechanisms, such as interference of regulation of DNA repair or integrity (inhibition of ligase and polimerase activity) [16,76,107].

It is also found that methylated forms of trivalent arsenic (MAs, DMAs) are the only arsenic compounds that can damage naked DNA and do not require exogenously added enzymatic or chemical activation systems [108]. Since less ionizable arsenic metabolites MMA(III), DMA (III) and MAs, DMAs are more toxic than inorganic arsenic, it is essential to reevaluate the hypothesis that methylation is the detoxification pathway for inorganic arsenic. MMA and DMA may be unusually capable of interacting with cellular targets, such as proteins and even DNA.

There is evidence that a large portion of arsenite-induced DNA strand breaks come from excision of oxidative DNA adducts and DNA-protein cross-links. Catalase, inhibitors of calcium, nitric oxide synthase, superoxide dismutase and myeloperoxidase may modulate arsenite-induced DNA damage. Arsenite induces DNA adducts through calcium-mediated production of peroxynitrite, hypochlorus acid and hydroxyl radicals [109,110].

Participation of oxygen free radicals in mutagenesis and carcinogenesis has been proposed by numerous investigators over the past 10 years [68]. The involvement of free radicals in ionizing radiation damage is well established. Recent findings suggest that oxidative processes may also play an important role in metal mutagenesis and carcinogenesis. Carcinogenic metals may act by altering the oxidative status of cells either directly (via Fenton-type reactions with endogenous hydrogen peroxide), or indirectly by affecting cellular antioxidative defenses such as glutathione. Neither superoxide anion($O_2$-), nor hydrogen peroxide ($H_2O_2$) reacts with DNA in the absence of metal ions.

The change of glutathione activity and cystein oxydation depends on radical-metal complexes. However, till now, there was no incontestable evidence that arsenic carcinogenicity and genotoxicity are based on oxidative stress conception. Only in last two – three years it has been shown that arsenite increases the level of superoxide-driven hydroxyl radicals in human-hamster hybrid cells, particularly in terms of reduced intracellular level of non-protein sulfhydryls (mainly glutathione). These data seem to provide convincing evidence that reactive oxygen species (hydroxyl radicals) play an important causal role in the genotoxicity of arsenical compounds in mammalian cells [20,51,79,97,111].

The oxidative stress may damage redox-sensitive signaling molecules, such as NO, S-nitrosothiols, AP-1, NF-kappaB, p53, p21ras, and others. This, in turn, may produce a variety of toxic effects of some metals (including arsenic), or even carcinogenesis. The attention of some authors is mainly focused on metal-induced signal transduction pathways leading to the activation of NF-kappaB, a transcription factor governing the expression of the earliest response genes involved in a number of human diseases, including cancers [112].

Studies on mutagenesis by metal compounds may be complicated by inducible tolerance mechanisms in some cells [68]. Metallothioneins (MT) are small cysteine-rich, sulfhydryl-rich, metal-binding proteins which bind a number of metals with high affinity and decrease its level in serum. So these are proteins that provide protection against metal toxicity. MT are induced by acute stress, hormones, metals and various organic compounds. Arsenicals have also been shown to induce MT in the liver, kidney, spleen, stomach, intestine, heart and lung. This induction profile is similar to that observed after Zn or Cd exposure. Its mechanism is still unknown.

Metallothioneins have been studied not only for its role in the detoxification of heavy metals but also in a variety of other physiological processes, including metabolism of essential metals Zn and Cu, cellular response to oxidative stress, and interaction with metallotherapeutic agents (Pt, Au). MT may also be involved in carcinogenesis by metals such as Ni (II), Cr (VI) and As (III). As (III) has a high affinity for thiols and appears to interact predominantly with DNA repair enzymes, but it is also a potent inducer

of MT biosynthesis. MT appears to be involved in a redox reaction with As (III) that generates oxidants, which are observed upon GSH depletion but are effectively scavenged by GSH at its normal cellular level [113–115].

The same biological features present other inducible proteins which may cause tolerance. Heat shock proteins are induced in human fibroblasts by As and other metals. Like metallothioneins, heat shock proteins have been postulated to play a role in the acquisition of tolerance to agents which induce their synthesis. The molecular mechanism of arsenic stress consists in the activation of mitogen-activated protein MAP-kinases, extracellular regulated kinase (ERK), c-jun terminal kinase (JNK), and p38, which induce the immediate early genes: c-fos, c-jun, and egr-1, responsible for the heat shock proteins synthesis and cell transformation [19,20,96].

Because of oxidative stress, the central component of heat shock response and arsenic-related effects occurs in various tissues [97], the sensitivity of these tissues to arsenic differs [116,117]. The kidney seems to be more sensitive, and the liver appears to be more protected by some of the antioxidant components, such as glutathione, glutathione-S-transferase and glucose-6-phosphate dehydrogenase. Arsenic may potentiate cadmium nephrotoxicity during the long-term, combined exposure, and intracellular metallothioneins play a role in decreasing nephropathy of this combined exposure.

The complete contradiction between the epidemiological and experimental evidence suggests that arsenic compounds **may act as cocarcinogens or comutagens** (enhancing agents) rather than primary carcinogens or mutagens [68]. Arsenite is comutagenic with UV in *E. coli*, for instance.

The mechanism of arsenite comutagenesis with alkylating agents is based on the reaction with DNA to form a variety of adducts, in which some favor primarily the base oxygens while the others favor the base nitrogens. These DNA adducts are excised by specific DNA glycosylases.

Some investigators suggest that the absence of normal p53 functioning, along with increased positive growth signaling in the presence of DNA damage, may result in defective DNA repair and account for the comutagenic effects of arsenite. Long-term, low dose exposure to arsenite result in increased expression of cyclin D1. That is the reason of p53-dependent increase in p21 expression, which is normally blocked after DNA damage, and cell transformation [16,76,99,107,118].

It is proposed that the specific co-clastogenic effects of arsenite may be mediated by its interference (inhibition) with DNA repair activities. This hypothesis was revealed following the observation that arsenite specifically potentiates chromosomal aberrations induced by a DNA cross-linking agent, 1,3-butadiene diepoxide, but does not affect the induction of sister chromatid exchanges under the same treatment conditions [68].

Many investigators suggest probable connection between the inorganic arsenic exposure and congenital malformations, low birth weight or fetal abortions [1]. These effects are, after all, not frequent at doses which do not cause toxic reactions in the mother organism.

Because of its widespread occurrence in animal and vegetable tissues, its toxicity, carcinogenicity and therapeutic use, arsenic should be studied extensively. According to Jager [92] further work, for example, is needed to determine the extent to which acquired or inherent human gene polymorphisms may contribute to interindividual variability in response to arsenic. Further studies are also required to compare such molecular events as methylation patterns of specific genes in human arsenic exposure-related tumors with the same tumor types from non-arsenic endemic areas. Continuous studies to isolate and characterize the activity of human and animal methyltransferases are needed. Finally, the focus of attention on the comparison between the effect of metabolism on toxicity and the effect of metabolism on carcinogenicity would also be desirable.

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