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SELECTED MECHANISMS OF GENOTOXIC EFFECTS OF INORGANIC ARSENIC COMPOUNDS

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Abstract. Chronic exposure to inorganic arsenic compounds is responsible for the prevalance of various tumors, as well as of other diseases. A major problem is the exposure to inorganic arsenic (i-As) in drinking water that affects millions of people, primarily in Asia and South America. In these regions, the concentration of arsenic in drinking water amounts to several thousand μg/l and considerably exceeds the standard of 50 μg/l, recommended by the US Environmental Protection Agency. It is interesting that not all populations are equally sensitive to i-As. Therefore, the existing standard should be verified and the environmentally safe i-As concentration should be established. Bearing this in mind, it would be helpful to know the mechanisms of toxicity of inorganic arsenic compounds.

In vitro and *in vivo* studies and examination of people exposed to high concentrations of i-As in drinking water show its genotoxicity. Inorganic As increases the frequency of micronuclei, chromosome aberrations and sister chromatid exchanges both in humans and in animals, but it does not induce point mutations. If arsenic does not affect DNA directly, then what is the mechanism of its toxicity? The results of various studies suggest that it may intensify toxic effects of other physical and chemical agents, especially by DNA repair inhibition. Besides, it is believed that inorganic arsenic compounds may cause changes in the cell redox potential and alter DNA methylation and phosphorylation of cell-cycle control proteins. Some data also suggest that i-As increases celluar proliferation and apoptosis. The purpose of this work is to present some views on cytotoxic mechanisms of inorganic arsenic compounds.

Key words:
Arsenic, Inorganic arsenic compounds, Genotoxicity, Mechanisms of genotoxicity

INTRODUCTION

Inorganic arsenic compounds are well recognised human carcinogens [1,2]. Epidemiological studies have shown that chronic exposure to inorganic arsenic (i-As) may lead to the development of many cancer types, including lung, skin, liver, kidney and urinary bladder cancers [3–6]. Exposure to high concentrations of i-As may also induce other diseases, such as skin pigmentation, hyperkeratosis of the palms of the hands and soles of the feet, or vascular disease [6–8].

There are many, mostly anthropogenic sources of inorganic arsenic in the environment. They include among others: smelting of nonferrous metals, burning of arsenic-containing coal, production and use of arsenical pesticides, herbicides, or insecticides, and glass manufacturing [1,3,4]. However, the main source of exposure to inorganic arsenic compounds is contaminated drinking water. This problem affects mainly the populations of Asia (Bangladesh, China, India, Thailand, Taiwan) [9] and South America (Chile, Argentina) [7]. The concentration of i-As in drinking water in these regions of the world dramatically exceeds the standard (50 μg/l) adopted in 1987 by the US Environmental Protection Agency (USEPA), and the number of the exposed comes to millions of people. In West Bengal (India) more than 30 million people are exposed to high con-

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centrations of i-As in drinking water, which amounts to 3400 μg/l [10]. Exceptionally high concentrations of inorganic arsenic compounds, of up to 4400 μg/l have been observed in artesian wells in some provinces of China – Shanxi and Xinjiang [9,11] and in Inner Mongolia (from 1800 μg/l up) [12].

It is striking that the different populations exposed to the similar i-As levels in drinking water vary in individual susceptibility. Moreover, the results of other studies show that some populations may be insensitive to carcinogenic effects of i-As, and in spite of high exposure, the increased cancer incidence has not been observed [13]. On the other hand, it has been demonstrated that over a one-thousand-year exposure of many generations of natives from northern Chile (Atacameno people) to i-As does not protect them from malignant diseases [7]. The various individual responses in populations may result from the presence of other factors promoting the carcinogenic process. They may include dietary, environmental (UV radiation), and genetic (polymorphism of certain enzymes, primarily methyltransferases) factors [14,15].

Identification and understanding of the mechanisms of the toxic effect of inorganic As compounds may be helpful in estimating cancer risk and preventing malignant and other diseases. There are many hypotheses on this issue that are not yet fully clarified and still arise a lot of controversy among researchers. Many studies have led to the evaluation of several basic theories. It is believed that inorganic As compounds do not affect DNA directly, form adducts with DNA, or induce DNA-protein crosslinks. Exposure to As *per se* does not cause point mutations, which are observed during simultaneous exposure to As and physical factors (UV radiation, X-radiation or gamma radiation), as well as to some chemical compounds (n-methyl-n-nitrosourea) [16]. This means that i-As is a co-mutagen and enhances mutagenic process of other agents. Some authors believe that the toxicity of inorganic As compounds results from the inhibition of the activity of DNA repair enzymes. This is caused mainly by physical and chemical properties of As and its ability to bind sulfhydryl groups present in the enzymes. The other data also suggest that inorganic As compounds are responsible for altered gene expression which is caused by the induction of oxidative stress [17,18] and/or disturbance of DNA methylation [19–23].

The increased expression of genes responsible for positive regulation of cell cycle and simultaneous inhibition of the expression of negative regulation genes was also observed during excessive cell proliferation caused by exposure to i-As. These genetic abnormalities usually lead to the development of malignancies. Thus i-As is undoubtedly carcinogenic, although some inorganic arsenic compounds, such as arsenic trioxide is used in China and India [24,25] as an anticarcinogenic drug.

The aim of this paper is to present the new data on carcinogenic and mutagenic properties of i-As and above all to systematize our knowledge about mechanisms of its genotoxic activity. We focused on the inhibition of DNA repair, disturbances in DNA methylation processes, oxidative stress induction, enhancement of cellular proliferation and induction of apoptosis.

CARCINOGENIC POTENTIAL OF INORGANIC AND ORGANIC As COMPOUNDS

For over one hundred years inorganic As compounds have been considered to be human carcinogens. Epidemiological data show that i-As causes lung, skin, liver, kidney and urinary bladder cancers. Lung cancer is associated with i-As exposure via inhalation, while exposure to i-As via ingestion induces skin, liver, kidney and bladder cancers [3,4]. It is believed that the most carcinogenic forms of As are inorganic compounds of trivalent and pentavalent As (As^{III} and As^{V}, respectively). Although inorganic As compounds are recognized as carcinogenic to humans [1,2], their carcinogenicity to animals is doubtful [26]. Only a few studies suggest that they may induce malignancies in animals. Ng et al. [27] showed that sodium arsenate administered to C57B1/6J mice induced at least one type of malignancy in about 40% of the animals.

Inorganic As undergoes a number of metabolic changes in the organism, leading to its decreased toxicity. These changes include reduction of As^{V} to As^{III} and methylation

Table 1. Genotoxic effects observed in the humans exposed to high concentrations of As in drinking water

Effect	Level of exposure to As	Cell type	References
MN	670 μg/l	Urinary bladder cells	37
CA	408 μg/l	Peripheral blood lymphocytes	36
MN	408 μg/l	Buccal cells and urothelial exfoliated cells	36
MN	593 μg/l	Buccal cells	13

of these forms to monomethylarsonic (MMA) and dimethylarsinic (DMA) acid.

The mechanism of As methylation proceeds according to the following scheme [15]:

$$H_2As^VO_4^- \Leftrightarrow H_3As^{III}O_3 \Rightarrow \underset{\text{MMA}}{CH_3As^VO_3^{2-}} \Leftrightarrow CH_3As^{III}O_2^{2-} \Rightarrow \underset{\text{DMA}}{(CH_3)_2As^VO_2^-}$$

Methylation is regarded as a detoxification process. Therefore, organic metabolites of As are probably harmless to people *in vivo*. The *in vitro* study of the human alveolar cells (L132) pointed out to considerable toxicity of DMA [28]. These data have been confirmed by investigations on animal models. The high concentrations of DMA (about 1500 mg/kg) induced single DNA breakage in the lung cells of male ICR mice [29], and the prolonged administration of DMA in drinking water developed urinary bladder cancer in F344 rats [30,31].

There are no complete literature data on toxicity of other organic As forms, such as arsenocholine and arsenobetaine, which occur mainly in the sea organisms. It seems that the amount of inorganic As released from these compounds during ingestion is rather small and probably harmless to humans [32].

MUTAGENIC AND GENOTOXIC PROPERTIES OF As

Inorganic As does not induce point mutations in bacteria or mammalian cells [16,33,34]. It may, however, cause multilocus deletions, which are rarely detected due to their lethal character. They were observed only in the specially selected cell models. Li and Rossman [16] found this type of mutations in locus *gpt* in the G12 cell lineage, containing *gpt* plasmid from *E. coli*. Other tests on hamster-human hybrid cells, containing human chromosome 11 encoding surface antigens (A_L cells), revealed that As induced mainly large chromosome mutations dependent on the size of the arsenic dose and mediated by reactive oxygen species (ROS) [35].

Inorganic As is genotoxic to animals and humans [36–39]. In laboratory animals exposed to sodium and potassium arsenate and to Fowler`s solution (arsenic oxide dissolved in potassium carbonate) at doses of 10 mg/kg, the increased frequency of micronuclei (MN) in bone marrow was observed [38]. In the culture of embryonic cells of Syrian hamster the frequency of chromosome aberrations (CA) and sister chromatid exchanges (SCEs) was increased in the presence of sodium arsenate and arsenite [39]. A higher level of genotoxic effects was also observed in people exposed to high concentrations of i-As in drinking water (Table 1).

EFFECTS OF CO-OPERATION OF As WITH SOME TOXIC AGENTS

Inorganic arsenic compounds at non-toxic concentrations have a comutagenic or cocarcinogenic character. They enhance toxic effects induced by other factors like X-radiation, UV radiation, alkylating agents (e.g. n-methyl--n-nitrosourea – MNU) [16,40–42]. It has been demonstrated that i-As strongly enhances mutagenic effects of UV radiation in *E. coli* [41]. The increase in the number of *E. coli* mutants was found to be directly proportional to the increase in As concentration for the same values of

UV radiation. Other authors report that non-toxic doses of sodium arsenite (5 μM for 24 h or 10 μM for 3 h) accompanied by exposure to MNU considerably increase the number of mutations in locus *hprt* in the cells of the V79 Chinese hamster [16]. Moreover, the results of another experiment revealed that co-exposure to As and Cd caused more severe damage in mice kidneys than separate exposure to each of these chemicals [43].

Toxic effects of inorganic As compounds depend, to a large extent, on a simultaneous exposure of the organism to different environmental agents. On the one hand, i-As supports the harmful effect of the above mentioned physical and chemical agents, but on the other, some chemical compounds counteract its toxicity. Compounds of selenium, antimony and zinc are among the examples of agents protecting from the toxic effects of i-As. Sodium selenite added to the culture of human lymphocytes considerably decreases the level of CA and SCEs induced by sodium arsenite [44]. Similar effects of simultaneous exposure to Se and As were observed in mice bone marrow, where a decreased level of CA was found [45]. It has also been demonstrated that trivalent antimony decreases the level of arsenic-induced MN. The presence of zinc in the organism decreases the extent of damage of peripheral vessels, thus lowering the risk of "blackfoot disease" [46]. However, this hypothesis has not been proved. The inhibition of the disease development may be due to other reasons as well. Therefore, it is necessary to carry out further investigations.

The mechanisms of interaction between inorganic As compounds and other factors remain unexplained. It is believed that As inhibits repair processes of DNA, which has been damaged by other than As toxic agents [40,42].

EFFECTS OF As ON CELLULAR FUNCTIONS

Inhibition of DNA repair

Data show that i-As inhibits the activity of enzymes which are involved in DNA repair by base excision repair (BER) and nucleotide excision repair (NER) [40,47]. DNA repair through BER and NER is a rather complex process and consists of several stages. They include: damage recognition, DNA strand cleavage, resynthesis of the damaged fragment and ligation. It is not known, however, at which stage the influence of inorganic As compounds is the strongest. It is judged that As inhibits the process of repair at its final stage – ligation. This hypothesis is supported by the findings indicating that As delays the ligation of DNA broken by As + MNU [16]. The delay of ligation of the repaired DNA fragments damaged by UV radiation was also observed in cultures of Chinese hamster ovary (CHO) cells [48] and in the culture of human fibroblasts [47]. Thus it may be concluded that i-As inhibits the activity of both polymerases and ligases. This probably results from high affinity of As to sulfhydryl groups of these enzymes, its binding to them and denaturation of enzymes. The study on the activity of purified DNA repair enzymes showed, however, that As^{III} or As^{V} at concentrations from 0,1 to 50 mM did not inhibit the activity of enzymes, even those with high content of sulfhydryl groups. Moreover, these concentrations induced an increase in the activity of DNA ligases I and III, DNA polymerase β. Similar results were obtained when the activity of DNA ligase extracts from unexposed AG06 keratinocyte cultures, treated with As^{III} and As^{V} after extraction, was compared with the activity of DNA ligase extracts from 24 h exposed cells. It was shown that the sensitivity of DNA ligases from untreated cells to As was not higher than that of purified enzymes. However, the decreased ligases activity in the exposed cell line was observed [49,50]. The lack of sensitivity of purified DNA repair enzymes and DNA ligases isolated from unexposed cells, and then treated As^{III} and As^{V} suggests that As does not influence directly the inhibition of repair enzymes, but affects ligation through changes in gene expression or post-translation modification of ligases, polymerases and other repair enzymes.

Disturbances in DNA methylation processes

It is believed that one of probable mechanisms of carcinogenic effect of inorganic As compounds is their influence on DNA methylation processes, which are necessary in the normal cell cycle regulation. This theory is based on the results of many studies indicating the relation between DNA methylation disturbances and the development of

malignancies. It has been observed that inorganic compounds of As^{III} may activate arsenite methyltransferases which, by using S-adenosyl-methionine (SAM) as a donor of methyl groups, decrease its concentration and cause DNA hypomethylation. Hence, the increased expression of protooncogenes [19–22] and simultaneously lowered expression of suppressor genes [51]. These results suggest that i-As may induce carcinogenesis through DNA hypomethylation and aberrant gene expression. Disturbances of methylation processes depend on the availability of SAM. Many studies demonstrate that deficiency of choline, methionine and folic acid in the diet of rats and certain strains of mice may induce carcinogenesis in hepatocytes [20]. These data are in agreement with the results of the studies on male rats fed methyl-deficient diet. It was found that the rats exhibited low level of DNA methylation and increased level of the mRNA fragments encoding *c-myc, c-fos* and *c-Ha-ras* genes, responsible for cancer induction [20,21]. The increase in expression of *c-myc* and *c-Ha-ras* was also observed in B6C3F1 mice [22]. Herman et al. [51] observed that inactivation of *p16* suppressor gene is often associated with its altered methylation and usually occurs in the most common types of human cancer. The disturbances in methylation processes and overexpression of protooncogenes are often accompanied by morphological changes. In male C57BL/6 mice fed a diet poor in substances supplying methyl radicals and simultaneously exposed to sodium arsenite hypertrophy of urinary bladder, loss of body mass and liver damage, manifested by a decreased number of hepatocytes and inflammatory infiltration, were observed [52].

Disturbances in the DNA methylation processes caused by i-As also lead to excessive methylation of DNA – hypermethylation. It comes from a partial inhibition of SAM-dependent methyltransferase activity, which results in the increase of SAM pool in the cell and excessive methylation of cytosines in DNA. *In vitro* studies on human lung cancer cells – A549 show that exposure to low doses of sodium arsenite and arsenate (0.08–2 μM and 30–300 μM, respectively) leads to hypermethylation of cytosines in the promoter region of *p53* gene. Cytosine hypermethylation may inhibit the transcription of the gene. As a result of *p53* gene blocking, the concentration of p53 protein in the cell decreases, followed by disturbances in the cell cycle control, DNA repair control and apoptosis control. On the other hand, it has been demonstrated that short-term exposure to i-As increases the concentration of p53 protein in cells [53]. As p53 protein is degraded by ubiquitin-dependent proteolysis, its increased level may be related to the blocking of this mechanism. This leads to the conclusion that As may disturb protein p53 proteolysis, and continuous overexpression of *p53* genes results in shutting down its expression by hypermethylation of its promoter. As a consequence, the amount of p53 protein is decreased and the cell cycle regulation processes are disturbed [34].

DNA methylation disturbances induced by inorganic arsenic compounds may, like inhibition of repair enzymes, directly affect cell physiology and lead to carcinogenesis. Similar carcinogenic effects may be caused by the induction of oxidative stress as a result of processes involving i-As.

Oxidative stress induction

The results of many studies demonstrate that many metals, including Cd, Cu, Fe and Pb may modulate the cellular redox state by changing the level of cell thiols, e.g. glutathione (GSH) [54]. The induction of oxidative stress in cells exposed to inorganic As compounds proceeds in a similar way.

Glutathione is an antioxidative compound, playing a key role in the process of i-As detoxification. It modulates the methylation reactions and stimulates excretion of DMA from the organism. Moreover, it reduces the harmful products of oxygen transformations according to the following scheme:

$$2GSH + R\text{-}O\text{-}OH \Rightarrow GSSG + H_2O + ROH$$

Exposure to i-As may lead to long-term changes in redox potential, and thus to changes in the control of cell functions which depend on this potential. Persistent changes in the cell cycle lead to the initiation of carcinogenic processes. The studies of cytotoxicity carried out on a culture of human AG06 keratinocytes showed that toxic effects of inorganic As compounds is strictly connected with the change in the level of reduced glutathione [49].

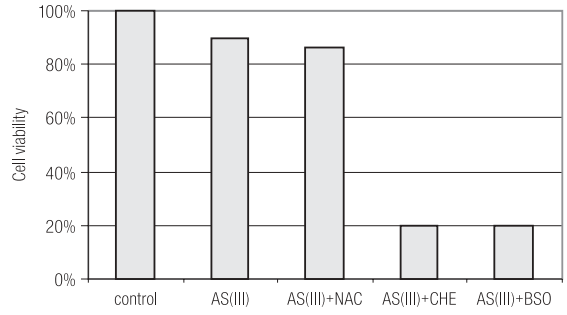

Fig. 1. Effect of As^{III} on the decrease of GSH concentration and viability of AG06 keratinocytes [49].
Keratinocytes were incubated for 24h with 10 mM NAC, 1.5 mM CHE and 250 μM BSO. Then they were exposed to 3 μM As for 48 h. Viability was determined by the method of neutral red dye uptake.

The sensitivity to As^{III} of AG06 keratinocytes with decreased GSH level by adding buthionine sulfoximine (BSO) or 2-chloroethanol (CHE) was over ten times higher. The effect of decreased viability was not so strong in the cells in which the level of GSH was increased by adding N-acetylcysteine (NAC) – GSH precursor (Fig. 1) [49]. Simultaneously, the study of activity of enzymes isolated from exposed cells did not reveal changes in the activity of glutathione reductase, although some authors believe that inhibition of its activity is responsible for decreasing the GSH level [40].

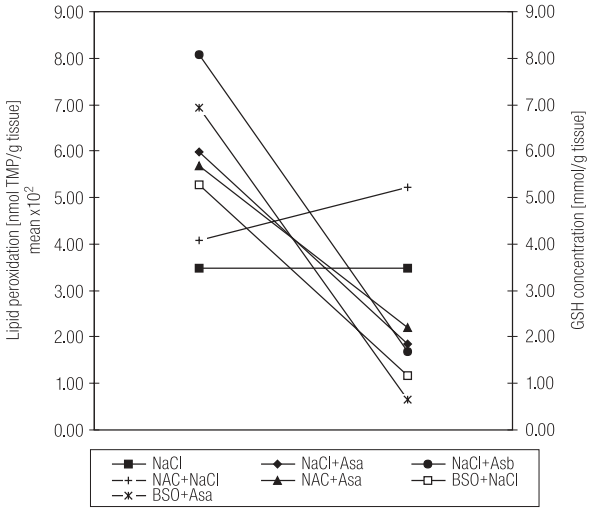

Fig. 2. Changes in GSH concentration and lipid peroxidation in the liver of rats exposed to sodium arsenite (Asa –14.8 mg/kg and Asb – 18.2 mg/kg) and in the presence of NAC or BSO [55].
Lipid peroxidation level was established by measuring the amount of TBA-RS (thiobarbituric acid reactive substances). TMP (1,1,3,3-tetramethoxypropane) was used as external reference. The level of TBA-RS was expressed in nmol TMP/g tissue.

The results of other studies show that a decrease in GSH concentration induced by i-As is related to the process of lipid peroxidation [55]. The decreased GSH level was observed, among others, in the rat liver, contributing to the increased lipid peroxidation. A similar tendency of GSH decrease and peroxidation increase was observed in the presence of NAC and BSO. Both the elevated and decreased GSH levels due to the presence of NAC and BSO, respectively, exhibit a falling tendency after exposure to As. At the same time, the decreased GSH concentration was, in both cases, accompanied by the increased lipid peroxidation (Fig. 2).

Furthermore, the data suggest that oxidative stress induced by i-As contributes to enhanced expression of *c-myc* oncogene and *GADD153* gene, which is responsible for the cell growth control, and for the inhibition of the cell cycle. The increase in *c-myc* expression was observed in the cultures of L6 rat myoblasts exposed to As. Moreover, *c-myc* expression increased rapidly when GSH concentration was decreased by adding BSO [17]. Guyton et al. [18] observed that arsenite increases *GADD153* gene expression, which is probably related to the decreased GSH concentration. At the same time, it is believed that ROS scavengers do not affect the expression of the above mentioned genes. This view is supported by the studies suggesting that the presence of ROS scavengers, such as o-phenanthroline, or mannitol, does not affect *GADD153* gene expression induced by As, whereas mannitol inhibits *GADD153* expression induced by H_2O_2 [40]. It follows that gene expression is modified by the effect of As on GSH and not on ROS.

Other studies indirectly indicate that inorganic As compounds induce oxidative stress. Superoxide dismutase in the culture of human lymphocytes incubated in the presence of As decreases the frequency of SCEs [56] and MN [57]. On the other hand, it is believed that i-As may play a protective role for the cell by inducing the increased concentration of proteins such as metallothionein [58], or heme oxygenase [59], which protect the cell from the effects of oxidative stress. However, the studies by Ng et al. [27] and Shimizu et al. [17] demonstrate that metallothionein does not protect cells from

cytotoxic effects of i-As and carcinogenesis induced by arsenic.

Enhancement of cellular proliferation

There is a lot of evidence to support the hypothesis that carcinogenic properties of inorganic As compounds result from modulation of cellular proliferation. It is believed that i-As affects the specific cell signal involved in cellular proliferation. Germolec et al. [60] observed that sodium arsenite at concentrations between 0.001 and 0.005 μM causes a considerable growth of the number of human keratinocytes, as evidenced by the increase in 3H-thymidine incorporation. Increased cellular proliferation was also observed in the cultures of human urinary bladder epithelial cells [61], as well as in the *in vivo* investigation on animals. In mice exposed to sodium arsenite, an increase in the number of skin epithelial cells and urinary bladder cells was observed [61]. The enhanced cellular proliferation was also found in female F344 rats exposed to DMA [62]. It has been observed that both in people and in animals enhanced cellular proliferation is accompanied by increased frequency of binding transcription factors such as AP-1 with DNA and the increased expression of genes *c-fos*, *c-jun*, *c-myc,* responsible for positive regulation of the cell cycle. The increased activation of AP-1-DNA binding in the presence of i-As may modulate *c-fos*, *c-jun* and *c-myc* expression, thus speeding up cellular proliferation. *In vitro* studies on both mice and human fibroblasts [63,64] showed that inorganic As compounds may modulate the mutagenic effect of certain growth factors which activate the transmission of extracellular proliferation signals to the cell nucleus by activation of receptor tyrosine kinase pathway (RTK). It is believed that i-As may disturb the positive and negative RTK pathway control. In the culture of C3H 10T1/2 fibroblasts after long-term exposure to sodium arsenite, the expression of E2F positive regulator genes and *myc* genes was increased while the expression of negative regulation MKP-1, $p27^{kip1}$ was decreased [64].

It has also been found that inorganic As compounds may affect the expression of growth factors, thus leading to the development of malignancies. Inorganic arsenic stimulates the expression of the tumor necrosis factor (TNFα), tumor growth factor (TGFα) and the granulocyte-macrophage colony stimulating factor (GM-CSF) in human keratinocyte cultures [60]. GM-CSF is involved not only in inflammatory reactions, but also in enhanced cellular proliferation. Besides, it has been observed that, like TGFα, GM-CSF is actively transcribed in the carcinogenic processes.

Other studies suggest that i-As affects the control of the cell cycle by activation of kinases, which catalyse the process of phosphorylation of proteins participating in the cell cycle regulation. The changes in kinase activity results in the disturbance of the cell cycle control and enhanced proliferation. Cavigelli et al. [65] found that sodium arsenite activates *c-jun* N-terminal kinase (JNK) in HeLa cells. This effect may cause the induction of protooncogenes *c-jun*/*c-fos* and activation of AP-1 transcription factor.

Induction of programmed cell death – apoptosis

The increased cellular proliferation caused by i-As is often associated with neoplastic changes in the organism. Paradoxically, arsenic trioxide at a proper dose (therapeutic concentration of As_2O_3 – 1–2 μM) is used as a cytostatic drug, inhibiting the oncogenic processes. Arsenic trioxide may induce clinical remission in patients with acute promyelocytic leukemia (APL) by inducing apoptosis, a programmed cell death [24,25,66,67]. The results obtained by Zhu et al. [25] show that As_2O_3 inhibits the growth of the examined malignant cell lines and decreases their viability (Table 2). These effects depend on the dose, exposure time and cellular susceptibility. This study reveals that the longer the cells were exposed to increasing doses of As_2O_3, the more their growth was inhibited and the more their viability was decreased. These effects are accompanied by morphological changes characteristic of apoptosis, such as shrinking cytoplasm, condensed chromatin and nuclear fragmentation with intact cell membrane.

At the same time, the degree of decreased mitochondrial transmembrane potential $\Delta\Psi_m$ was checked [25]. Earlier studies demonstrated that $\Delta\Psi_m$ plays a significant role in the process of apoptosis [68]. Lowered $\Delta\Psi_m$ potential is probably associated with the opening of the mitochon-

Table 2. Survival and cell growth inhibition of different cell lineages* after exposure to arsenic trioxide in dose- and time-dependent manner [25]

Exposure time	As_2O_3 concentration	Effects	
		Inhibition of cell growth (%) range	Cell viability (%) range
1 day	1 μM	0–27	87–100
	2 μM	0–55	74–92
3 days	1 μM	15–64	60–98
	2 μM	31–93	25–87
5 days	1 μM	25–81	39–98
	2 μM	41–100	0–80

* The cell lineages used for the investigation included human cancer cell lineages: ALL – acute lymphocytic leukemia, CLL – chronic lymphocytic leukemia and lymphoma cell lineages:
Nalm-6 – lymphocytic lineages from pre-B – cell ALL,
Namalwa, Raji – Burkitt`s lymphoma cells,
BJAB – B-cell lymphoma,
su-DHL-4 – follicular B-cell lymphoma with t(14,18) chromosomal translocation,
Molt-4, Jurkat – T-lymphocytic lineages from T-cell ALL,
SKW-3 – T-lymphocytic lineages from T-cell CLL.

drial permeability transition pores (MPT) or mitochondrial channels, which connect the inner and the outer mitochondrial membranes. It is believed that i-As decreases $\Delta\Psi_m$ potential by thiol oxidation of structural proteins present in MPT pores. The formed disulphide bonds lower $\Delta\Psi_m$ and open MPT pores. The change in the mitochondrium transmembrane potential is related to the lowered ATP level. It has been observed that in susceptible cells, ATP concentration is twice as low as in the cells in which As_2O_3 does not induce any changes. These data confirm earlier results of Yih et al. [69] who found lowered ATP levels in HeLa S3 cells.

It seems that apoptosis induced by inorganic As compounds is caused mainly by prolonged cell cycle, and not by the growth arrest in mitotic stage [25]. Different results were obtained by Huang et al. [70], who prove that apoptosis in HeLaS3 cells exposed to sodium arsenite is caused by the fact that the cells are arrested in mitosis. According to them, this is probably due to a delayed cyclin B degradation. It is well known, that on the one hand, cyclin B is necessary to initiate mitosis, but on the other, its degradation is needed to proceed to anaphase and complete the cell cycle. It has been shown that in cells exposed to sodium arsenite, degradation of cyclin B is considerably delayed in comparison with the process occurring in the control cells. Even 6 h after reincubation of cells in a medium without As, the level of cyclin B was still high, while in the control cells after 2 h cyclin B was completely degraded. Li and Broome [71] suggest that leukemic cells are arrested in mitosis due to interaction of As_2O_3 with tubulin.

Data also reveal that As_2O_3 may induce apoptosis in leukemic cells as a result of decreased expression of *bcl-2* gene [24,67] and modulation of promyelocitic leukemia-retinoic acid receptor α chimeric protein (PML-RARα) [24,66]. In the studies on the human leukemic cells lines (NB_4) carried out in the presence of As_2O_3, a decreased expression of *bcl-2* gene whose product has an antiapoptotic character and is responsible for the defense of the cell against apoptosis, was observed. The decrease of the amount of bcl-2 protein, in the cell leads to the induction of apoptosis. This effect was observed in a microscopic analysis of morphologically changed cells and in an electrophoretic analysis of characteristic ladder structure. It is believed that interactions of bcl-2 protein with other proteins, especially with bax protein also play a key role in the regulation of apoptosis processes. The combination of antagonistic proteins neutralizes antiapoptotic properties of bcl-2, thus inducing programed cell death.

A decreased chimeric PML/RARα protein concentration is also responsible for apoptosis initiated by i-As. Among other properties, PML/RARα plays the role of apoptosis

suppressor in myelocytic cells. It has been shown that in the presence of As_2O_3, the amount of PML/RARα decreases and cellular apoptosis proceeds.

CONCLUSIONS

The proposed models of toxic effect of inorganic As compounds on organisms are rather diversified. It is believed that i-As does not react directly with DNA and does not cause mutations. However, it induces chromosome damage and may enhance mutagenesis caused by other toxic agents, especially by inhibiting DNA repair. Many researchers demonstrate that As inhibits DNA repair at the stage of ligation. It probably does not cause directly the inhibition of ligase activity, but inhibits ligation through changes in gene expression or post-translation enzyme modification. Inorganic arsenic may also disturb the process of DNA repair control by inhibiting genes encoding proteins which regulate the DNA repair, such as p53 protein. Some reports point out to inhibition of *p53* gene expression as a result of hypermethylation of the promoter region of this gene [23]. On the other hand, i-As may cause global hypomethylation of DNA, disturbances in the activity of methyltransferases and hyperexpression of protooncogen *c-myc*. Overexpression of protooncogen *c-myc* and *GADD153* was also observed in the cells in which GSH concentration was lowered in the presence of As. The decrease of GSH concentration and induced oxidative stress contribute to the increased lipid peroxidation.

Exposure to i-As is also associated with the increased cellular proliferation, the expression of oncogens *c-myc, c-jun, c-fos,* and the increased frequency of AP-1 – DNA binding. Enhanced proliferation induced by i-As is associated with the modulation of RTK pathway regulators, the increase of expression of growth factors and the change of kinase activity. The effects observed in other studies, including lowered ATP level, inhibited cyclin B degradation and decreased expression of antiapoptotic protein genes bcl-2 and PML/RARα are related to apoptosis induction.

In a multistage process of carcinogenesis, synergistic and/or antagonistic effects of various dietary and environmental factors also has to be considered. There is evidence that these factors (UV radiation and some chemical compounds) with i-As may considerably increase the risk of malignant disease, or to the contrary, neutralize the toxic effect of this metalloid. It is not known which of the described mechanisms of i-As action prevail in the inducing cancer development. They probably compete with each other. Disturbances of DNA repair, DNA methylation, changes in redox potential or hyperexpression of oncogenes in the long-term exposure to As cause certain abnormalities in the cell. In consequence, they may lead to the development of carcinogenic process.

A detailed analysis of the mechanisms of harmful effect of inorganic As compounds would allow to predict the risk of cancer, malignant and other diseases, prevent them and perhaps facilitate their treatment. Moreover, the knowledge of the mechanisms of i-As toxicity and the estimation of toxic doses would help determine the safe level of As in the environment. In the meantime, a large number of issues which still remain unclear underlines the need for further studies, both *in vivo* and *in vitro.*

REFERENCES

1. IARC. *Arsenic and arsenic compounds. Some metals and metallic compounds. IARC Monographs on the Evaluation of Carcinogenic Risk to Humans*. Lyon, France: International Agency for Research on Cancer; 1980. p. 39–141.
2. IARC. *Arsenic*. *Overall evaluation of carcinogenicity: an updating of IARC monographs*. Lyon, France: International Agency for Research on Cancer; 1987, Suppl. 7. p. 100–6.
3. Chen CJ, Chen CW, Wu MM, Kuo TL. *Cancer potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking water*. Br J Cancer 1992; 66: 888–92.
4. Smith AH, Hopenhayn-Rich C, Bates MN, Goeden HN, Herz--Piccioto I, Duggan H, et al. *Cancer risk from arsenic in drinking water*. Environ Health Perspect 1992; 97: 259–67.
5. Morales KH, Ryan L, Kuo TL, Wu MM, Chen CJ. *Risk of internal cancers from arsenic in drinking water*. Environ Health Perspect 2000; 108 Suppl 7: 655–61.
6. Tsai SM, Wang TS, Ko YC. *Mortality for certain diseases in areas with high levels of arsenic in drinkig water*. Arch Environ Health 1999; 54 Suppl 3: 186–93.

7. Smith AH, Arroyo AP, Mazumder DNG, Kosnett MJ, Hernandez AL, Beeris M, et al. *Arsenic-induced skin lesions among Atacameno people in Northern Chile despite good nutrition and centuries of exposure*. Environ Health Perspect 2000; 108 Suppl 7: 617–20.
8. Col M, Col C, Soran A, Sayli BS, Ozturk S. *Arsenic related Bowen`s disease, palmar keratosis, and skin cancer*. Environ Health Perspect 1999; 107 Suppl 8: 687–9.
9. Chen CJ, Hsu LI, Tseng CH, Hsueh YM, Chiou HY. *Emerging epidemics of arseniasis in Asia*. In: Chappell WR, Abernathy CO, Calderon RL, editors. *Arsenic Exposure and Health Effects*. 1st ed. Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo: Elsevier Science; 1999. p. 113–21.
10. Guha Mazumder DN, De BK, Santra A, Dasgupta J, Ghosh N, Roy BK, et al. *Chronic arsenic toxicity: epidemiology, natural history and treatment*. In: Chappell WR, Abernathy CO, Calderon RL, editors. *Arsenic Exposure and Health Effects*. 1st ed. Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo: Elsevier Science; 1999. p. 335–47.
11. Sun GF, Dai GJ, Li FJ, Yamauchi H, Yoshida T, Aikawa H. *The present situation of chronic arsenism and research in China*. In: Chappell WR, Abernathy CO, Calderon RL, editors. *Arsenic Exposure and Health Effects*. 1st ed. Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo: Elsevier Science; 1999. p. 123–5.
12. Ma HZ, Xia YJ, Wu KG, Sun TZ, Mumford JL. *Human exposure to arsenic and health effects in Bayingnormen, Inner Mongolia*. In: Chappell WR, Abernathy CO, Calderon RL, editors. *Arsenic Exposure and Health Effects*. 1st ed. Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo: Elsevier Science; 1999. p. 127–31.
13. Aposhian HV, Arroyo A, Cebrian ME, Del Razo LM, Hurlbut KM, Dart RC, et al. *DMPS-arsenic challenge test. I: Increased urinary excretion of monomethylarsonic acid in humans given dimercaptopropane sulfonate*. J Pharmacol Exp Ther 1997; 282 Suppl 2: 192–200.
14. Vahter M, Concha G, Nermell B, Nilsson R, Dulout F, Natarajan AT. *A unique metabolism of inorganic arsenic in native Andean women*. Eur J Pharmacol 1995; 293: 455–62.
15. Vahter M. *Variation in human metabolism of arsenic*. In: Chappell WR, Abernathy CO, Calderon RL, editors. *Arsenic Exposure and Health Effects*. 1st ed. Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo: Elsevier Science; 1999: 267–79.
16. Li JH, Rossman TG. *Mechanism of comutagenesis of sodium arsenite with n-methyl-n-nitrosourea*. Biol Trace Elem Res 1989; 21: 373–81.
17. Shimizu M, Hochandel JF, Fulmer BA, Waalkes MP. *Effect of glutathione depletion and metallothionein gene expression on arsenic-induced cytotoxicity and c-myc expression in vitro*. Toxicol Sci 1998; 45: 204–11.
18. Guyton KZ, Xu Q, Holbruk NJ. *Induction of the mammalian stress response gene GADD 153 by oxidative stress: role of AP-1 element*. Biochem J 1996; 314: 547–54.
19. Zhao CQ, Young MR, Diwan BA, Coogan TP, Wallkes MP. *Association of arsenic-induced malignant transformation with DNA hypomethylation and aberrant gene expresion*. Proc Natl Acad Sci USA 1997; 94: 10907–12.
20. Dizik M, Christman JK, Wainfan E. *Alternetions in expression and methylation of specific genes in livers of rats fed cancer promoting methyl-deficient diet*. Carcinogenesis 1991; 12 Suppl 7: 1307–12.
21. Christman JK, Sheikhnejad G, Dizik M, Abileah S, Wainfan E. *Reversibility of changes in nucleic acid methylation and gene expression induced in rat liver by severe dietary deficiency*. Carcinogenesis 1993; 14 Suppl 4: 551–7.
22. Hsieh LL, Wainfan E, Hoshina S, Dizik M, Weinstein IB. *Altered expression of retrovirus-like sequences and celluler onkogenes in mice fed methyl-deficient diets*. Cancer Res 1989; 49: 3795–9.
23. Mass MJ, Wang L. *Arsenic alters cytosine methylation patterns of the promoter of the tumor supressor gene p53 in human lung cells: a model for mechanism of carcinogenesis*, Mutat Res 1997; 386: 263–77.
24. Chen GQ, Zhu J, Shi XG, Ni JH, Zhong HJ, Si GY, et al. *In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB_4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-RARα/PML proteins*. Blood 1996; 88 (3):1052–61.
25. Zhu XH, Shen YL, Jing YK, Cai X, Jia PM, Huang Y, et al. *Apoptosis and growth inhibition in malignant lymphocytes after treatment with arsenic trioxide at clinically achievable concentrations*. J Natl Cancer Inst 1999; 91: 772–8.
26. Huff J, Chan P, Nyska A. *Is the human carcinogen arsenic carcinogenic to laboratory animals*? Toxicol Sci 2000; 55: 17–23.
27. Ng JC, Seawright AA, Qi L, Garnett C M, Chiswell B, Moore MR. *Tumours in mice induced by exposure to sodium arsenate in drinking water*. In: Chappell WR, Abernathy CO, Calderon RL, editors. *Arsenic Exposure and Health Effects*. 1st ed. Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo: Elsevier Science; 1999. p. 217–23.

28. Yamanaka K, Hayashi H, Tachikawa M, Kato K, Hasegawa A, Oku N, et al. *Metabolic methylation is a possible genotoxicity-enhancing process of inorganic arsenics*. Mutat Res 1997; 394: 95–101.
29. Yamanaka K, Hasegawa A, Sawamura R, Okada S. *Dimethylated arsenics induce DNA strand breaks in lung via the production of active oxigen in mice*. Biochem Biophys Res Commun 1989; 165 Suppl 1: 43–50.
30. Wei M, Wanibuchi H, Yamamoto S, Li W, Fukushima S. *Urinary bladder carcinogenicity of dimethylarsinic acid in male F344 rats*. Carcinogenesis 1999; 20 Suppl 9: 1873–6.
31. Wanibuchi H, Wei M, Yamamoto S, Li W, Fukushima S. *Carcinogenicity of an organic arsenic, dimethyl-arsinic acid and related arsenicals in rat urinary bladder*. Proc Am Assoc Cancer Res 1999; 40: 349.
32. Gebel T. *Confounding variables in the environmental toxicology of arsenic*. Toxicology 2000; 144: 155–162.
33. Rossman TG, Stone D, Molina M, Troll W. *Absence of arsenite mutagenicity in E. coli and Chinese hamster cells*. Environ Mutagen 1980; 2: 371–9.
34. Rossman TG. *Arsenite genotoxicity maybe mediated by interference with DNA damage-inducible signaling.* In: Chappell WR, Abernathy CO, Calderon RL, editors. *Arsenic Exposure and Health Effects*. 1st ed. Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo: Elsevier Science; 1999. p. 233–41.
35. Hei TK, Liu SX, Waldren C. *Mutagenicity of arsenic in mammalian cells: role of reactive oxygen species*. Proc Natl Acad Sci USA 1998; 95: 8103–8.
36. Gonsebatt ME, Vega L, Salazar AM, Montero R, Guzman P, Blas J, et al. *Cytogenic effects in human exposure to arsenic*. Mutat Res 1997; 386 Suppl 3: 219–28.
37. Biggs ML, Kalman DA, Moore LE, Hopenhayn-Rich C, Smith MT, Smith AH. *Relationship of urinary arsenic to intake estimates and a biomarker of effect, bladder cell micronuclei*. Mutat Res 1997; 386: 185–95.
38. Tinwell H, Stephens SC, Ashby J. *Arsenite as the probable active species in the human carcinogenicity of arsenic: mouse micronucleus assays on Na and K arsenite, orpiment, and Fowler`s solution*. Environ Health Perspect 1991; 95: 205–10.
39. Barrett JC, Lamb PW, Wang TC, Lee TC. *Mechanisms of arsenic-induced cell transformation.* Biol Trace Elem Res 1989; 21: 421–9.
40. Simenova PP, Luster MI. *Mechanism of arsenic carcinogenicity: genetic or epigenetic mechanisms?* J Environ Pathol Toxicol Oncol 2000; 19 Suppl 3: 281–6.
41. Rossman TG. *Enhancement of UV-mutagenesis by low concentrations of arsenite in E. coli*. Mutat Res 1981; 91: 207–11.
42. Snyder RD, Davis GF, Lachmann PJ. *Inhibition by metals of X-ray and ultraviolet-induced DNA repair in human cells*. Biol Trace Elem Res 1989; 21: 389–98.
43. Liu J, Liu Y, Habeebu SM, Waalkes MP, Klaassen CD. *Chronic combined exposure to cadmium and arsenic exacerebates nephrotoxicity, particularly in metallothionein-I/II null mice*. Toxicology 2000; 147: 157–66.
44. Beckman L, Nordenson I. *Interaction between some common genotoxic agents*. Hum Hered 1986; 36: 397–401.
45. Biswas S, Talukder G, Sharma A. *Prevention of cytotoxic effects of arsenic by short-term dietary supplementation with selenium in mice in vivo*. Mutat Res 1999; 441: 155–60.
46. Gebel T. *Suppresion of arsenic-induced chromosome mutagenicity by antimony*. Mutat Res 1998; 412: 213–8.
47. Hartwig A, Groblinghoff UD, Beyersmann D, Natarajan AT, Filon R, Mullenders LHF. *Interaction of arsenic(III) with nucleotide excision repair in UV-irradiated human fibroblasts*. Carcinogenesis 1997; 18 Suppl 2: 399–405.
48. Lee-Chen SF, Yu CT, Jan KY. *Effects of arsenite on DNA repair of UV-irradiated Chinese hamster ovary cells*. Mutagenesis 1992; 1: 51–5.
49. Snow ET, Hu Y, Yan CC, Chouchane S. *Modulation of DNA repair and glutathione levels in human keratinocytes by micromolar arsenite*. In: Chappell WR, Abernathy CO, Calderon RL, editors. *Arsenic Exposure and Health Effects*. 1st ed. Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo: Elsevier Science; 1999. p. 243–51 .
50. Hu Y, Su L, Snow TE. *Arsenic toxicity is enzyme specific and its effects on ligation are not caused by the direct inhibition of DNA repair enzymes*. Mutat Res 1998; 408: 203–18.
51. Herman JG, Merlo A, Mao L, Lapidus RG, Issa JPJ, Davidson NE. *Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers*. Cancer Res 1995; 55: 4525–30.
52. Okoji RS, Leininger J, Froines JR. *Subchronic toxicity study of sodium arsenite in methyl-deficient male C57BL/6 mice*. In: Chappell WR, Abernathy CO, Calderon RL, editors. *Arsenic Exposure and Health Effects*. 1st ed. Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo: Elsevier Science; 1999. p. 225–32.
53. Salazar AM, Ostrosky-Wegman P, Menedez D, Miranda E, Garcia-Carranca A, Rojas E. *Induction of p53 protein expression by sodium arsenite*. Mutat Res 1997; 381: 259–65.

54. Stohs SJ, Bagchi D. *Oxidative mechanisms in the toxicity of metal ions*. Free Radic Biol Med 1995; 18: 321–36.

55. Ramos O, Carrizales L, Yanez L, Mejia J, Batres L, Ortiz D, et al. *Arsenic increased lipid peroxidation in rat tissues by mechanism independent of glutathione*. Environ Health Perspect 1995; 103 Suppl 1: 85–8.

56. Nordenson I, Beckman L. *Is the genotoxic effect of arsenic mediated by oxygen free radicals?* Hum Hered 1991; 41: 71–3.

57. Gurr JR, Liu F, Lynn S, Jan KY. *Calcium-dependent nitric oxide production is involved in arsenic-induced micronuclei*. Mutat Res 1998; 416: 137–48.

58. Albores A, Koropatnick J, Cherian MG, Zelazowski AJ. *Arsenic iduces and enhances rat hepatic methallothionein production in vivo*. Chem Biol Interact 1992; 85: 127–40.

59. Keyse SM, Tyrrell RM. *Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UV radiation, hydrogen peroxide, and sodium arsenite*. Proc Natl Acad Sci USA1989; 86: 99–103.

60. Germolec DR, Spalding J, Boorman GA, Wilmer JL, Yoshida T, Simenova PP, et al. *Arsenic can mediate skin neoplasia by chronic stimulation of keratinocyte-derived growth factor*. Mutat Res 1997; 386: 209–18.

61. Simenova PP, Wang S, Toriuma W, Kommineni V, Matheson J, Unimye N, et al. *Arsenic mediates cell proliferation and gene expression in the bladder epithelium: association with activating protein-1 transactivation*. Cancer Res 2000; 60: 3445–53.

62. Cohen SM, Arnold LL, St. John MK, Cano M. *Evaluation of cell proliferative activity in the rat urinary bladder after feeding high doses of cacodilic acid*. In: Chappell WR, Abernathy CO, Calderon RL, editors. *Arsenic Exposure and Health Effects*. 1st ed. Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo: Elsevier Science; 1999. p. 253–62.

63. Trouba KJ, Glanzer JG, Vorce RL. *Wild-type and Ras-transformed fibroblasts display differential mitogenic responses to transient sodium arsenite exposure*. Toxicol Sci 1999; 50: 72–81.

64. Trouba KJ, Wauson EM, Worce RL. *Sodium arsenite-induced dysregulation of proteins involved in proliferative signaling*. Toxicol Appl Pharmacol 2000; 164: 161–70.

65. Cavigelli M, Li WW, Lin A, Su B, Yoshioka K, Karin M. *The tumor promoter arsenite stimulates AP-1 activity by inhibiting a JNK phosphatase*. EMBO J 1996; 15 Suppl 22: 6269–79.

66. Shao W, Fanelli M, Ferrara FF, Riccioni R, Rosenauer A, Davidson K, et al. *Arsenic trioxide as an inducer of apoptosis and loss of PML/RARα protein in acute promyelocytic leukemia cells*. J Natl Cancer Inst 1998; 21: 124–33.

67. Zhang W, Ohnishi K, Shigeno K, Fujisawa S, Naito K, Nakamura S, et al. *The induction of apoptosis and cycle arrest by arsenic trioxide in lymphoid neoplasms*. Leukemia 1998; 12: 1383–91.

68. Zamzami N, Hirsch T, Dallaporta B, Petit PX, Kroemer G. *Mitochondrial implication in accidental and programmed cell death: apoptosis and necrosis*. J Bioenerg Biomembr 1997; 29 Suppl 2: 185–93.

69. Yih LH, Huang H, Jan KY, Lee TC. *Sodium arsenite induces depletion and mitochondrial damage in HeLa cells*. Cell Biol Int Rep 1991; 15 Suppl 3: 253–64.

70. Huang SC, Huang CYF, Lee TC. *Induction of mitosis-mediated apoptosis by sodium arsenite in HeLa S3 cells*. Biochem Pharm 2000; 60: 771–80.

71. Li YM, Broome JD. *Arsenic targets tubulins to induce apoptosis in myeloid leukemia cells.* Cancer Res 1999; 59: 776–80.

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