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Arsenic: toxicity, oxidative stress and human disease

K. Jomova,[a] Z. Jenisova,[a] M. Feszterova,[a] S. Baros,[a] J. Liska,[b] D. Hudecova,[c] C. J. Rhodes[d] and M. Valko[c]*

ABSTRACT: Arsenic (As) is a toxic metalloid element that is present in air, water and soil. Inorganic arsenic tends to be more toxic than organic arsenic. Examples of methylated organic arsenicals include monomethylarsonic acid [MMA(V)] and dimethylarsinic acid [DMA(V)]. Reactive oxygen species (ROS)-mediated oxidative damage is a common denominator in arsenic pathogenesis. In addition, arsenic induces morphological changes in the integrity of mitochondria. Cascade mechanisms of free radical formation derived from the superoxide radical, combined with glutathione-depleting agents, increase the sensitivity of cells to arsenic toxicity. When both humans and animals are exposed to arsenic, they experience an increased formation of ROS/RNS, including peroxyl radicals ($ROO^{\bullet}$), the superoxide radical, singlet oxygen, hydroxyl radical ($OH^{\bullet}$) via the Fenton reaction, hydrogen peroxide, the dimethylarsenic radical, the dimethylarsenic peroxyl radical and/or oxidant-induced DNA damage. Arsenic induces the formation of oxidized lipids which in turn generate several bioactive molecules (ROS, peroxides and isoprostanes), of which aldehydes [malondialdehyde (MDA) and 4-hydroxy-nonenal (HNE)] are the major end products. This review discusses aspects of chronic and acute exposures of arsenic in the etiology of cancer, cardiovascular disease (hypertension and atherosclerosis), neurological disorders, gastrointestinal disturbances, liver disease and renal disease, reproductive health effects, dermal changes and other health disorders. The role of antioxidant defence systems against arsenic toxicity is also discussed. Consideration is given to the role of vitamin C (ascorbic acid), vitamin E (α-tocopherol), curcumin, glutathione and antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase in their protective roles against arsenic-induced oxidative stress. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: arsenic; oxidative stress; free radicals; ROS; RNS; human disease; antioxidants

INTRODUCTION

Arsenic is the 33rd element of the Periodic Table of the chemical elements, and while it is classified formally as a metalloid, meaning that it displays some properties of both a metal and a nonmetal, it is frequently also referred to as a metal and in the context of toxicology as a *heavy* metal (Mandal and Suzuki, 2002). Arsenic exists in nature in three allotropic forms, α (yellow), β (black), γ (grey), of the metallic state and in a number of ionic forms. The most common oxidation numbers of arsenic are +5, +3 and −3, in which the element is able to form both inorganic and organic compounds both in the environment and within the human body (Orloff *et al.*, 2009). In combination with other elements such as oxygen, sulfur and chlorine, the element is referred to as *inorganic arsenic* and as combined with hydrogen and carbon as *organic arsenic*. Since most arsenic compounds lack colour or smell, the presence of arsenic is not immediately obvious in food, water or air, thus presenting a serious human health hazard given the toxic nature of the element. Indeed, the very name arsenic is synonymous with poison, in consequence of its long and nefarious history (Mandal and Suzuki, 2002). Arsenic is ubiquitous in nature and its abundance ranks twentieth in the Earth's crust, fourteenth in seawater and twelfth in the human body.

SOURCES AND ROUTES OF EXPOSURE TO ARSENIC

Arsenic trioxide (As_2O_3) is the most prevalent inorganic arsenical found in air, while a variety of inorganic arsenates (AsO_4^{3-}) or arsenites (AsO_2^{-}) occur in water, soil or food (Magalhaes, 2002; Chou *et al.*, 2007). In consequence of its widespread use in the microelectronics industry, gallium arsenide (GaAs) is an inorganic arsenic compound which may also impact adversely on human health.

The largest source of arsenic and other metals is usually food, of which the main dietary forms are seafood, rice, mushrooms and poultry (Jones, 2007; Petroczi and Naughton, 2009; Nepusz *et al.*, 2009; Smedley and Kinniburgh, 2002). While there is more arsenic *per se* in seafood, this is mostly in an organic form called arsenobetaine which is much less harmful than others. Mostly, arsenic poisoning occurs through industrial exposure, from contaminated wine or moonshine, or by malicious administration.

Very recently, it has been reported that traditional Chinese herbal products, deliberately fortified with arsenic for therapeutic

*Correspondence to: M. Valko, Faculty of Chemical and Food Technology, Slovak Technical University, SK-812 37 Bratislava, Slovakia.
E-mail: marian.valko@stuba.sk

[a]Department of Chemistry, Faculty of Natural Sciences, Constantine The Philosopher University, SK-949 74 Nitra, Slovakia

[b]Institute of Histology and Embryology, Medical Faculty of Comenius University, SK-811 08 Bratislava, Slovakia

[c]Faculty of Chemical and Food Technology, Slovak Technical University, SK-812 37 Bratislava, Slovakia

[d]Fresh-Lands Environmental Actions, 88 Star Road, Caversham, RG4 5BE, UK

purposes, may represent a serious health hazard (Martena *et al.*, 2010). Mass spectrometry has revealed that, of 292 tested samples sold in Dutch market, 20% significantly exceeded safety levels not only of arsenic but also of lead and mercury. It has been concluded that traditional herbal preparations of Chinese and Tibetian medicine require strict control by local authorities.

Colour pigments that are used in the cosmetic industry in the production of eye-shadows frequently contain toxic elements, including arsenic (Sainio *et al.*, 2000). The skin of the eyelids is very delicate and the application of eye-shadows may produce eczemas. In addition, arsenic particles can be water soluble and therefore may undergo percutaneous absorption through the wet skin. When it eneters the circulatory system via percutaneous absorption, at high concentrations, arsenic may represent a potential risk of carcinogenesis. Based on the available toxicology data, it has been recommended that cosmetic products should contain less than 5 ppm of metal impurities.

As contained in water, soil or food, ingested arsenic may quickly enter the human body. When air containing arsenic dusts is breathed in, the majority of the dust particles settle onto the lining of the lungs (Chen *et al.*, 2006). Very little internal exposure to arsenic occurs via the material passing through the skin into the body, and so there is little risk of arsenic poisoning posed by this route.

The majority of arsenic enters the body in the trivalent inorganic form As(III) via a simple diffusion mechanism (Cohen *et al.*, 2006). Only a small amout of pentavalent inorganic arsenic can cross cell membranes via an energy-dependent transport system, after which it is immediately reduced to trivalent arsenic.

Both organic and inorganic forms of arsenic leave the body in urine and thus most inorganic arsenic will be expelled after several days, although some will remain for a number of months or even longer (Aposhian *et al.*, 2000a, b). The majority of organic arsenic is expelled more rapidly and usually within several days.

Groundwater contamination by arsenic and other metals has impacted severely on the health of the populations of various regions in the world. Some of the most profound examples of contamination by arsenic occur in Bangaldesh and West Bengal, in India, where it has been discovered that almost 43 million people have been drinking water that is laden with arsenic (Chowdhury *et al.*, 2000). To place this in perspective, the WHO recommended limit for arsenic in water is 10 μg l^{-1} (WHO factsheet no. 210, May 2001), while concentrations in the range 50–3200 μg l^{-1} have been measured (Bhattacharya *et al.*, 2003). Table 1 summarizes the levels of arsenic in human tissues and urine collected from residents from the arsenic-contaminated areas.

Table 1. Levels of As in human urine and tissues of the residents from the arsenic-polluted areas[a]

Country/town or region	Concentration of arsenic/source of sample	References
UK/Glasgow		
	0.650 μg g^{-1} (hair)	Raie (1996)
	0.048 μg g^{-1} (liver)	
	0.015 μg g^{-1} (spleen)	
	0.007 μg g^{-1} (lung)	
Germany/Nürnberg, Saxony		
	5.5 ng g^{-1} (lung)	Kraus *et al.* (2000)
	7.58 μg g^{-1} (urine)	Gebel *et al.* (1998)
Turkey/Ismir		
	4.23 μg l^{-1} (breast milk)	Ulman *et al.* (1998)
Mexico/Hermosa		
Concentration of As in water:		
9 μg l^{-1}	10.26 μg per day (urine, 24 h)	Wyatt *et al.* (1998)
15 μg l^{-1}	10.54 μg per day (urine, 24 h)	
30 μg l^{-1}	25.18 μg per day (urine, 24 h)	
USA/Fort Valley		
	11.6 μg l^{-1} (urine, randomly collected)	Hewitt *et al.* (1995)
	11.0 μg l^{-1} (urine, 24 h)	
	0.78 μg g^{-1} (hair)	
Middleport		
	15.1 μg l^{-1} (urine) (children <7 years)	Tsuji *et al.* (2005)
	15.7 μg l^{-1} (urine) (children <13 years)	
	15.7 μg l^{-1} (urine) (adults)	
Spain/Catalania		
	<0.05 μg g^{-1} (lung)	Garcia *et al.* (2001)
	<0.05 μg g^{-1} (bone)	
	<0.05 μg g^{-1} (kidney)	
	<0.05 μg g^{-1} (liver)	
India/West Bengal		
	7.32 μg g^{-1} (finger nails)	Mandal *et al.* (2003)
	4.46 μg g^{-1} (hair)	

[a]Adapted from Chou *et al.* (2007).

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TOXICITY OF ARSENIC

Inorganic arsenic includes arsenite [As(III)] and arsenate [As(V)] and can be either methylated to form monomethylarsonic acid [MMA(V)] or dimethylated as in dimethylarsinic acid [DMA(V)]. The metabolism of inorganic arsenic involves a two-electron reduction of pentavalent arsenic to trivalent arsenic, mediated by glutathione, followed by oxidative methylation to form pentavalent organic arsenic (Fig. 1; Hughes, 2002).

Inorganic arsenic tends to be far more toxic than organic arsenic (Shi *et al.*, 2004; Valko *et al.*, 2005). Arsenic is toxic to the majority of organ systems, the most sensitive target organ being the kidney (Cohen *et al.*, 2006; see Table 1). The extent of arsenic poisoning depends on various factors such as dose, individual susceptibility to arsenic and the age of the affected individuals. While chronic arsenic exposure affects the vascular system and causes hypertension and cardiovascular disease, acute arsenic toxicity may cause cardiomyopathy and hypotension. The most common neurological effect of long-term arsenic toxicity is peripheral neuropathy and the gastrointestinal effects are manifested by toxic hepatitis accompanied by increased levels of liver enzymes.

Trivalent inorganic arsenic inhibits pyruvate dehydrogenase by binding to the sulfydryl groups of dihydrolipoamide, resulting in a reduced conversion of pyruvate to acetyl coenzyme A (CoA), while both citric acid cycle activity and production of cellular ATP are decreased (Bergquist *et al.*, 2009). Trivalent arsenic inhibits numerous other cellular enzymes through sulfydryl group binding. It also inhibits the uptake of glucose into cells, gluconeogenesis, fatty acid oxidation and further production of acetyl CoA. Significant to oxidative stress is that trivalent arsenic inhibits the production of glutathione, which protects cells against oxidative damage (Miller *et al.*, 2002).

In part, the toxicity of pentavalent inorganic arsenic is due to its conversion to trivalent arsenic, from which the toxic effects proceed as outlined above. At a more significant and specific level, pentavalent arsenic emulates inorganic phosphate and replaces phosphate in glycolytic and cellular respiration pathways (Hughes, 2002). Uncoupling of oxidative phosphorylation occurs because the normal high-energy phosphate bonds are not formed; e.g. in the presence of pentavalent arsenic, adenosine diphosphate (ADP) forms ADP-arsenate instead of ATP with the absence of the high-energy ATP phosphate bonds.

The methylation of inorganic arsenic has been considered to be a detoxification mechanism (Aposhian, 1997). However, recent experimental results have documented the presence of trivalent intermediates, monomethylarsonous acid [MMA(III)] and dimethylarsinous acid [DMA(III)] in the urine of humans exposed to drinking water containing high levels of inorganic arsenic (Cohen *et al.*, 2006). These trivalent intermediates are structurally different from the pentavalent compounds and are more reactive and more carcinogenic.

BIOMARKERS OF ARSENIC

Quantification of the Exposure to Arsenic

Measurements of the level of arsenic in blood, urine, hair and nails have all been used as biological indicators of exposure to arsenic (Vahter, 1983). Since arsenic is metabolized from blood within a period of several hours (Tam *et al.*, 1979), the measurement of blood arsenic levels is not a good indicator of long-term exposure of individuals to arsenic.

On the basis that no correlation was found between the level of arsenic in the blood and the level of arsenic in the drinking water of residents in several communities in the USA, where water levels ranged from about 6 to 125 μg l^{-1} (Valentine *et al.*, 1979), it was concluded that measurement of blood arsenic is not a reliable marker for arsenic exposure. We may note that typical background values of blood concentration for Arsenic in nonexposed individuals are <1 μg l^{-1} (Heydorn, 1970), while blood levels in acutely toxic and fatal cases may be 1000 μg l^{-1} or even greater (Driesback, 1980).

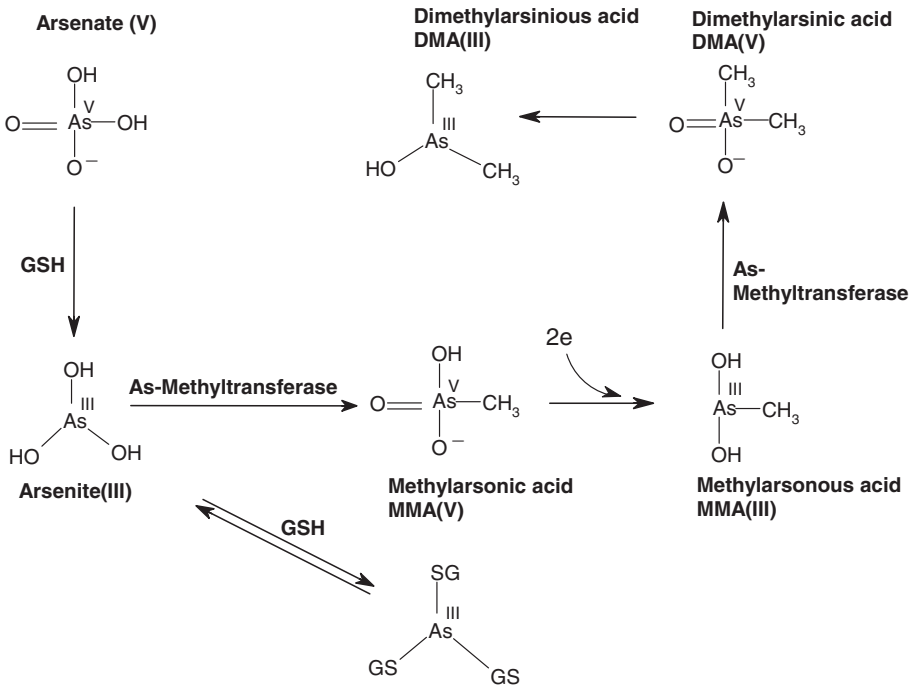

Figure 1. The metabolism of inorganic arsenic.

Since the majority of arsenic is absorbed from the lungs or the gastrointestinal tract and excreted in the urine, normally within 1–2 days, measurement of urinary arsenic levels is generally considered as the most reliable marker of acute arsenic exposure (Polissar *et al.*, 1990). Indeed, such measurements are found to correlate well with exposures of populations living near industrial point sources of arsenic (see for example Milham and Strong, 1974).

Biomarkers of the Effect Caused by Arsenic

Arsenic is known to influence the activity of a number of enzymes, in particular the group of enzymes responsible for heme synthesis and degradation (Woods and Fowler, 1978) and activation of heme oxygenase (Sardana *et al.*, 1981). Menzel and coworkers (Menzel *et al.*, 1998) have also examined the *in vitro* induction of human lymphocyte heme oxygenase 1 (HO1) as a biomarker of arsenite exposure. Arsenite was observed to induce *de novo* synthesis of HO1 in human lymphoblastoid cells, but it has not been determined whether the same response is induced *in vivo*.

Animal tests have shown that arsenic poisoning increased urinary levels of uroporphyrin, coproporphyrin and bilirubin (Albores *et al.*, 1989). These tests have also been shown to be applicable to human subjects (García-Vargas and Hernández-Zavala, 1996). Hence, altered urinary levels of these heme-related compounds could serve as a sensitive biomarker of the effect of arsenic.

ARSENIC AND OXIDATIVE AND NITROSATIVE STRESS

Many mechanistic studies of arsenic toxicity have suggested that reactive oxygen species and reactive nitrogen species are generated during inorganic arsenic metabolism in living cells (Shi *et al.*, 2004).

Arsenic induces morphologic changes in mitochondrial integrity and a rapid decline of mitochondrial membrane potential. Mitochondrial alterations are considered to be primary sites where an uncontrolled random formation of superoxide anion radical occurs. Cascade mechanisms of free radical formation derived from the superoxide radical combined with a decrease in cellular oxidant defence by treatment with glutathione-depleting agents results in an increased sensitivity of cells to arsenic toxicity (Cohen *et al.*, 2006; Valko *et al.*, 2005). Experimental results based on both *in vivo* and *in vitro* studies of arsenic-exposed humans and animals suggest the possible involvement of increased formation of peroxyl radicals ($ROO^{\bullet}$), superoxide anion radical ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), dimethylarsenic radical [$(CH_3)_2As^{\bullet}$], blood nonprotein sulfydryls and/or oxidant-induced DNA damage (Flora *et al.*, 2007). The exact mechanism responsible for the generation of all these reactive species has yet to be fully elucidated, but some studies have proposed the formation of intermediary arsine species.

An interesting route to H_2O_2 production was proposed to involve the oxidation of As(III) to As(V) which, under physiological conditions, results in the formation of H_2O_2 (Valko *et al.*, 2005 and references therein).

$$H_3AsO_3 + H_2O + O_2 \rightarrow H_3AsO_4 + H_2O_2 \ (\Delta_r G^{\theta} = -40.82 \text{ kcal mol}^{-1}) \quad (1)$$

The above reaction is spontaneous and exergonic with an estimated standard reaction free energy change for H_2O_2 formation of –40.82 kcal mol^{-1} (–170.87 kJ mol^{-1}).

Hydrogen peroxide is not a free radical species; however, when an organism is overloaded by iron (as in the conditions of haemochromatosis, haemolytic anemias and haemodialysis), the high cellular concentrations of 'free available iron' may have deleterious effects, as is demonstrated by the participation of Fe (II) in the decomposition of hydrogen peroxide (Fenton reaction), generating highly reactive hydroxyl radicals:

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\bullet}OH + OH^{-} \text{ (Fenton reaction)} \quad (2)$$

The hydroxyl radical is a highly reactive ROS with a half-life shorter than 1 ns in an aqueous environment (Valko *et al.*, 2007). Thus when it is produced *in vivo* it reacts in regions close to its site of formation. The formation of $^{\bullet}OH$ in the vicinity of DNA might lead to this radical reacting with DNA bases or with the deoxyribose backbone of DNA to produce modified (damaged) bases or strand breaks. The majority of the hydroxyl radicals generated *in vivo* arise from the metal catalysed decomposition of hydrogen peroxide, according to the Fenton reaction (Naughton *et al.*, 1993).

In addition to reactive oxygen species, arsenic exposure can initiate the generation of reactive nitrogen species (RNS). Several contradictory results describing arsenic-induced production of $NO^{\bullet}$ have been reported, one of which concluded that there was no arsenic-induced increase in $NO^{\bullet}$ generation in hepatocytes and human liver cells, which inhibited inducible NO synthase gene expression in cytokine-stimulated human liver cells and hepatocytes (Hughes, 2002; Flora *et al.*, 2008; Germolec *et al.*, 1996). However, in another study, arsenite was said to inhibit inducible NO synthase gene expression in rat pulmonary artery smooth muscle cells (Kodavanti *et al.*, 1998). A third study with low levels of arsenite (<5 μM) similarly recorded no change in intracellular concentration of Ca(II), nor any $NO^{\bullet}$ generation, according to results from EPR spectroscopy (Barchowsky *et al.*, 1999).

GENOTOXICITY OF ARSENIC

There have been a large number of *in vitro* and *in vivo* studies made, devoted to determining the genotoxicity of inorganic arsenicals (Yamanaka *et al.*, 2004; Cohen *et al.*, 2006). *In vitro* studies on human fibroblasts, leukocytes, lymphocytes and hamster embryo cells have shown that arsenic induces chromosomal aberrations and sister chromatid exchange (Helleday *et al.*, 2000). Similar studies using human, mouse and hamster cells explored a potential enhancement of DNA damage, DNA repair enhancement or the inhibition of DNA synthesis.

Studies of humans have detected a higher than average incidence of chromosomal aberrations in peripheral lymphocytes, after both inhalation exposure (Nordenson *et al.*, 1978) and oral exposure (Nordenson *et al.*, 1979). These studies must be interpreted with caution, since in most cases, there were only a small number of subjects and influences from exposure to other chemicals could also affect the results.

Investigations of genotoxic effects of ingested arsenic in Taiwanese residents have yielded mixed results, possibly due to the different types of cells being examined and the different exposure levels experienced by the populations studied. Arsenic-related skin cancer has shown an accompanying much

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higher rate in p53 mutations in comparison with those found in UV-induced skin cancer (Hsu *et al.*, 1999).

Occupational exposure of arsenic among workers in a glass plant in India whose levels of blood arsenic were five times higher than in the control group was reported to lead to increased DNA damage in leukocytes (Vuyyuri *et al.*, 2006).

An increased occurence of chromosomal abnormalities was detected in rats given oral doses of sodium arsenate (4 mg As kg^{-1} per day) for 2–3 weeks (Datta *et al.*, 1986). However, no increase in chromosomal aberrations was detected in bone marrow cells or spermatogonia from mice given sodium arsenite for a period of 2 months (Poma *et al.*, 1987). These studies suggest that ingested arsenic may cause chromosomal effects, but the data really are too limited to extract any firm conclusions.

The genotoxicity of organic arsenic has also been thoroughly investigated (see for example Kuroda *et al.*, 2004). DMA causes several genotoxic effects, including single strand DNA breaks, the formation of apurinic and apyrimidinic sites, an enhancement in oxidative stress as documented by oxidation of DNA bases, formation of DNA–protein crosslinks and chromosomal aberrations (Kitchin, 2001). Clastogenic effects of arsenic have been attributed to the high afinity of arsenic to sulfydryl groups of proteins. Several tests indicate that not only DMA but also roxarsone (3-nitro, 4-hydroxyphenylarsonic acid) may be able to cause mutations and DNA strand breaks. *In vitro* studies with MMA did not find significant increases in the occurrence of chromosome aberrations, mutations or unscheduled DNA synthesis. In addition, an increased number of DNA strand breaks was detected in lung and other tissues of mice and rats given oral doses of ~1500 mg kg^{-1} DMA (Okada and Yamanaka, 1994); this effect appeared to be related to the formation of some active oxygen species. Since the breaks were largely repaired within 24 h, the relevance related to any health risk is uncertain.

A study of p53 mutations in arsenic-related skin cancers from patients in Taiwan exposed to arsenic from drinking water found a high rate of p53 mutations and different types of p53 mutations compared with those seen in UV-induced skin cancers; similar results have been found in mice (Salim *et al.*, 2003).

While some animal studies have shown an increased incidence of chromosomal abnormalities in rats given oral doses of sodium arsenate for several weeks (Datta *et al.*, 1986), other sudies did not confirm any consistent increase in chromosomal aberrations detected in bone marrow cells or spermatogonia in mice given sodium arsenite (Poma *et al.*, 1987). Thus the available data are too limited to draw a solid conclusion.

The most extensively studied DNA lesion is the formation of 8-OH-G, one of the major products of DNA oxidation, which originates from the reaction of hydroxyl radical with guanine (Fig. 2; Valko *et al.*, 2006). 8-OH-G is a sensitive genotoxic marker of oxidatively damaged DNA. Associations with increased urinary 8-OH-G concentrations have been seen also for arsenic exposure.

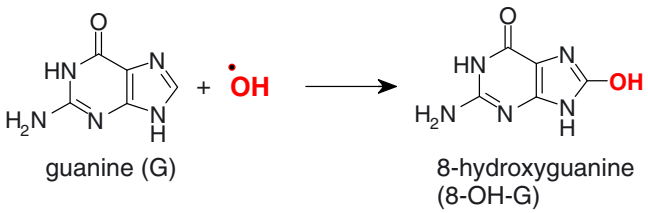

Figure 2. Reaction of guanine base (G) with hydroxyl radical.

As described above, arsenic is metabolized via methylation. A high concentration in urine, of the monomethylated As metabolite, methylarsonic acid (MMA), which is a susceptibility factor for As-induced toxicity, including carcinogenicity, has been correlated with similarly high urinary concentrations of 8-OH-G (Hu *et al.*, 2006). Interestingly, based on clinical trials, for a wide range of As exposure with urinary-As concentrations up to 1200 μg l^{-1}, accepting the known pro-oxidative effects of As, the association of 8-OH-G with urinary-As was shown to be weaker than that for moderate exposure to cadmium. Thus, 8-OH-G may not be as sensitive a biomarker for As-induced oxidative stress as it is for Cd and for oxidative stress induced by other metals (Engström *et al.*, 2010).

ARSENIC AND HUMAN DISEASE

Arsenic-induced genotoxicity may involve an alteration of the integrity of the cellular genetic material by oxidants or free radical species. Many recent studies have provided experimental evidence that arsenic-induced generation of free radicals and oxidative stress can cause cell damage and cell death through activation of oxidative sensitive signalling pathways (Fig. 3; De Vizcaya-Ruiz *et al.*, 2009). Arsenic exposure has been linked with various types of cancer (Miller *et al.*, 2002), cardiovascular diesase (Navas-Acien *et al.*, 2005), diabetes (Díaz-Villaseñor *et al.*, 2007), neurological disorders (Vahidnia *et al.*, 2007) and dermal effects (Cohen *et al.*, 2006).

Dermal Disease

Chronic exposure to arsenic leads to the development of lesions on the skin, including hyperkeratosis and hyperpigmentation, often used as diagnostic criteria for arsenicosis. (McCarty *et al.*, 2007). Dermal effects following the exposure to arsenic are hallmarks of the early stages of arsenic poisoning. Arsenic-induced cancers may appear later, sometimes taking several decades to develop symptoms (Lage *et al.*, 2006).

Workers exposed to inorganic arsenic in the air suffered from contact dermatitis and mild dermal irritation. Similar dermal effects (hyperkeratosis and hyperpigmentation) have been observed by the oral route of exposure among farmers in Taiwan who had been drinking arsenic-contaminated well water. The author of the trial stated that occurence of dermal lesions was found to increase with dose (Tseng, 1977). Low, medium and high exposure levels corresponded to doses of 0.0008, 0.014, 0.038 and 0.065 mg As kg^{-1} per day, respectively.

There is limited data accumulated for humans exposed to organic arsenic in air. Keratosis was observed in female workers in a chemical plant who were exposed to aersanilic acid (0.065 mg m^{-3}; Chou *et al.*, 2007). Animal studies have shown that rats exposed to DMA (6 mg m^{-3}) developed erythematous lesions on the feet and ears.

Oral exposure to organic arsenicals (MMA) with respect to dermal effects in humans has not been studied. Animal studies using rats and mice reported no histological skin alterations following chronic exposure to MMA.

Cancer

Arsenic is a pernicious environmental carcinogen, and leads mainly to cancers of the skin, albeit that there is epidemiogolical evidence for lung, bladder, liver and kidney cancers being

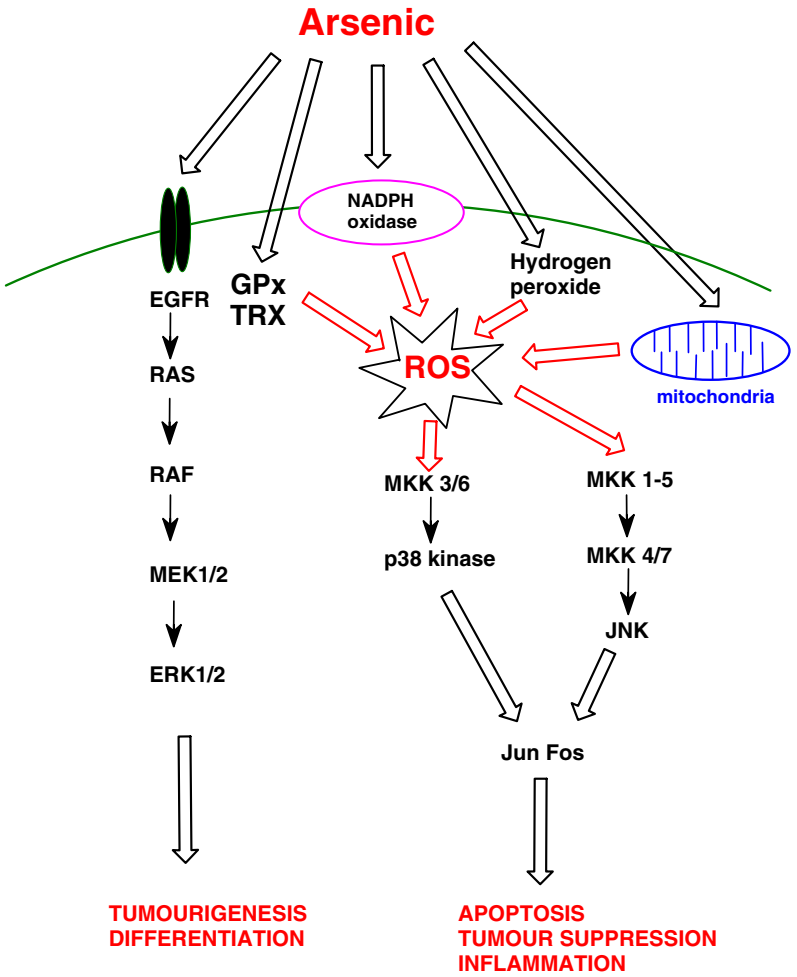

Figure 3. Arsenic-mediated cell signalling (EGFR, epidermal growth factor receptor; Ras, GPx, glutathione peroxidase; Trx, thioredoxin; JNK, c-Jun N-terminal kinases, MKK, Mitogen-activated protein kinase kinases).

caused by exposure to arsenic (Rossman, 2003). It is thought that the mechanism by which these cancers originate may involve the promotion of oxidative stress by arsenic compounds, in which the antioxidant capacity of the living organism is overwhelemed by ROS (reactive oxygen species), resulting in molecular damage to proteins, lipids and most significantly DNA (Liu *et al.*, 2001).

Trivalent arsenic has been demonstrated to exhibit a greater toxicity than the corresponding pentavalent forms, in addition to a far more pronounced ability to release iron from the iron-storage protein ferritin (Salnikow and Zhitkovich, 2008). Free iron may catalyse the decomposition of hydrogen peroxide via the Fenton reaction, thereby forming the reactive hydroxyl radical which can cause DNA damage.

A large number of epidemiological trials have reported that inhalation exposure to inorganic arsenic increases the risk of lung cancer. An increased incidence of lung cancer has been reported among workers exposed primarily to arsenic trioxide dust, (Wall, 1980; Welch *et al.*, 1982); however, the incidence of lung cancers has also been observed among workers exposed primarily to arsenate (Bulbulyan *et al.*, 1996). The latter study also reported an increased risk of stomach cancers among workers who had been exposed to the highest concentrations of arsenic.

Quantitative dose–response data obtained from copper smelters provide the most compelling evidence that arsenic is responsibile for the development of lung cancer (Mazumdar *et al.*, 1989). The conclusions of these studies are nonetheless limited by the confounding exposure to other chemicals, such as sulfur dioxide, and those from cigarette smoking.

An interesting link between arsenic exposure and smoking has been found in a nested case–control analysis of 102 lung cancer cases along with 190 controls. It was found that the incidence of lung cancer increases with increasing arsenic exposure in both smokers and nonsmokers (Järup and Pershagen, 1991). Histological examinations found an increase in several types of lung tumours, indicating that arsenic does not specifically increase the incidence of one particular type of lung cancer.

In addition to lung cancer, other minor types of nonrespiratory cancers associated with inhalation exposure to inorganic arsenic have been reported. Enterline and coworkers (Enterline *et al.*, 1995) found a significantly increased mortality due to cancer of the large intestine and bone cancer. It should be noted, however, that the apparent increase in the risk of bone cancer was based on a very small number of observations.

Other studies have shown an increase in nonmelanoma skin cancers as a result of exposure from a Slovakian coal-burning

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power plant (Pesch *et al.*, 2002) and an increase in the risk of stomach cancer among workers exposed to the highest average arsenic concentrations at a Russian fertilizer plant (Bulbulyan *et al.*, 1996).

Human exposure to inorganic arsenic is associated with an increased risk of dermal malignancies (Pi *et al.*, 2008); however, arsenic has been found to act as a cofactor in the development of skin tumours in combination with ultraviolet (UV) irradiation or exposure to phorbol esters. This suggests that the events associated with arsenic-induced dermal carcinogenesis may be distinct from other target tissues.

Long-term arsenic exposure has been reported to cause a malignant transformation of human keratinocytes *in vitro* (Pi *et al.*, 2008). Arsenic-transformed cells were found to show a weakened Nrf2 (nuclear factor E2-related factor 2)-mediated antioxidant defence, activation coupled with apoptotic resistance, increased expression of casein kinase 2 (CK2) and elevated basal Nrf2 activity. Arsenic-induced apoptotic resistance and weakened antioxidant response may therefore be critical steps in development of dermal cancer after exposure to arsenic.

It is generally accepted that methylated organic arsenicals are significantly less toxic than the inorganic forms (Kitchin, 2001). Methylation is part of a natural process of enhanced excretion of arsenic and appears to be a detoxification mechanism for inorganic arsenic. However, the process of methylation may lead to formation of reactive and carcinogenic trivalent methylated arsenicals (MMAIII and DMAIII; see above) (Cohen *et al.*, 2001, 2002). Thus the process of methylation of inorganic arsenic may provide a toxic pathway and both trivalent methylated arsenic (monomethylarsonous and dimethylarsinous acids) may possess harmful biological activity.

Animal studies did not show any signs of a prospective carcinogenic effect of MMA(V) (reviewed in Cohen *et al.*, 2006). The absence of carcinogenic effect of MMA(V) is in agreement with the negligible amount of DMA(V) formed from exogenously administered MMA(V) (Hughes and Kenyon, 1998).

In contrast to MMA(V), DMA(V) has been shown to induce bladder tumours in rats administered at high doses (100 pm) in the diet for 2 years (Gemert and Eldan, 1998). Since DMA(V) and MMA(V) are stored in the lumen of the bladder, this organ is much more prone to carcinogenic transformation than liver and kidney because of the reductive process leading to formation of DMA(III) and MMA(III) (Kitchin, 2001).

DMA(V) has been documented by a number of studies to act as a cancer promoter in co-administration of other tumorigenic compounds (Wanibuchi *et al.*, 2004; Kitchin, 2001). DMA(V) significantly increased the incidence of bladder, kidney, thyroid gland and liver cancer (Yamamoto *et al.*, 1995). DMA(V) has been reported to act as a skin tumour promoter in mice, accelerating the induction of 7,12-dimethylbenz[a]anthracene (DMBA)-induced skin tumours in mice (Morikawa *et al.*, 2000).

Rats fed with DMA(V) (200 ppm in water) exhibited an increased urinary concentration of DMA(III) in a dose-dependent manner (Okina *et al.*, 2004). This study has also shown that the levels of MMA(III) and DMA(III) may play a significant role in the toxicity and carcinogenicity towards the bladder induced by DMA(V).

It has been proposed that DMA(III) is an unstable metabolite and is stabilized through the formation of a DMA(III)–GSH conjugate, which is responsible for the toxic effect of DMA(III) (Styblo *et al.*, 2000). The cytotoxicity of trivalent (MMA(III), DMA (III) and also of As(III) arsenic can be suppressed by the application of antioxidants. Positive effects have been found following application of vitamin C and N-acetylcysteine, which preferentially interacts with trivalent arsenicals via its sulfydryl group (Wei *et al.*, 2005). Interestingly, melatonin and trolox did not show a protective effect against arsenic toxicity. The mechanism of genotoxicity of the DMA(III) does not involve direct interaction with DNA, but is most probably achieved indirectly via formation of ROS (Kitchin and Ahmad, 2003). ROS formation activates the transcription factors (e.g. AP-1, *c-fos* and NF-kB), and oversecretion of proinflammatory and growth promoting cytokines, resulting in increased cell proliferation and ultimately carcinogenesis.

The exact molecular mechanism of carcinogenesis caused by arsenic is still under investigation by many researchers. Currently accepted molecular mechanisms of arsenic toxicity involve genetic and epigenetic changes, the role of oxidative stress, enhanced cell proliferation and modulation of gene expression. Arsenic is known to induce the hypoxia signalling pathway (Galanis *et al.*, 2009). Treatment of DU145 prostate cancer cells with arsenite induced HIF-1α expression in a concentration- and time-dependent manner, whereas the level of HIF-1β remained unaffected. The VEGF (vascular endothelial growth factor) protein level was also elevated. ROS formation was linked with the activation of the PI3K/Akt pathway and the subsequent induction of HIF-1α and VEGF.

Cardiovascular Effect

While serious and adverse effects on the cardiovascular system following oral exposure to arsenic are well known, there is some evidence from epidemiological studies that the cardiovascular system may also be affected by inhaled inorganic arsenic (Navas-Acien *et al.*, 2005; States *et al.*, 2009).

Among the more profound effects on the heart from long-term exposure to arsenic are altered myocardial depolarization and cardiac arrhythmias (Cullen *et al.*, 1995; Mumford *et al.*, 2007). Long-term, low/medium-level exposure has been shown to cause mild damage to the vascular system; however, severe hypertrophy of the ventricular wall was observed after an acute exposure to a high (93 mg) concentration of arsenic (Quatrehomme *et al.*, 1992).

Wang and coworkers (Wang *et al.*, 2003) found an increased incidence of disease in the blood vessels in Taiwanese populations living in areas with arsenic-polluted wells (>0.35 mg l^{-1}). In addition, attempts to assess the relative risks for stroke and peripheral arterial disease have been conducted. However, there are methodologic limitations for the interpretation of the observed data and it would hence appear sensible to make such studies of the effect of arsenic on the cardiovascular system a research priority.

In another ecological study conducted in the USA, a significant increase in the number of deaths from arteriosclerosis, aneurysm and other related diseases were found in the areas in which the drinking water contained arsenic concentrations >20 µg l^{-1} (Engel and Smith, 1994). No significant cardiovascular effects were noted after acute ingestion of monosodium methylarsenate (1714 mg kg^{-1}).

Vascular endothelium is well known to regulate the release of various mediators such as nitric oxide, angiotensin-II, endothelin-1, adhesion molecules, cytokines and other similarly acting species (Balakumar and Kaur, 2009; Quyyumi, 1998). NO has been considered to be a major mediator released from endothelium. It has vasodilatory and anti-inflammatory

properties, and inhibits platelet adhesion and aggregation, smooth muscle cell proliferation and migration. Exposure of endothelial cells to sodium arsenite induces a decline in the integrity of vascular endothelium and endothelial cytotoxicity by inactivating protein kinase B/Akt and eNOS, so reducing the generation and bioavailability of NO, and increasing the oxidative stress and subsequently decreasing the endothelium-dependent vasorelaxation (Balakumar and Kaur, 2009).

Arsenic has been shown to induce atherosclerosis by increasing mRNA transcripts of growth factors including granulocyte–macrophage colony-stimulating factor, transforming growth factor-*α* and the inflammatory cytokinelike tumour necrosis factor-*α* (Germolec *et al.*, 1997; Kitchin, 2001). Experimental studies of the effect of arsenic on the vascular system have shown that oxidized lipids are present in all stages of atherogenesis which in turn generate several bioactive molecules (e.g. ROS, peroxides and isoprostanes), of which aldehydes are the major end products. Malondialdehyde (MDA) and 4-hydroxy-*trans*-2-nonenal (HNE) are the most abundant aldehydes generated from the oxidation of LDL and possess mutagenic and carcinogenic properties (Valko *et al.*, 2005, 2007). Protein adducts of MDA and HNE have been detected in atherosclerotic lesions of experimental animals and humans.

Evidence from a large number of studies indicates that inflammation plays a pivotal role in atherosclerotic plaque formation. Vascular cells generate chemokines and proinflammatory cytokines including monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6) and tumour necrosis factor *α*. This suggests that As-induced inflammation could be an important risk factor for atherosclerosis (Tsou *et al.*, 2005).

Hypertension is another disorder associated with increased arsenic exposure (Yang *et al.*, 2007). Arsenic-induced hypertension has been explained by an enhanced myosin light-chain phosphorylation and an increase in calcium-sensitization in blood vessels. Disruption of the antioxidant defence system leads to elevated systolic blood pressure. The possible mechanisms of arsenic-induced atherosclerosis, vascular endothelial dysfunction and hypertension are shown in Fig. 4.

Gastrointestinal Disturbances

Inorganic arsenicals

Clinical signs of gastrointestinal irritation, including nausea, vomiting, diarrhoea and abdominal pain, are observed in all cases of short-term high-dose and longer-term lower-dose exposures to inorganic arsenic (Uede and Furukawa, 2003; Vantroyen *et al.*, 2004). Haemorrhagic gastrointestinal lesions have been reported in animal studies. For example, a monkey fed with 6 mg As kg^{-1} per day for approximately 1 month was found, upon necropsy, to have died of acute inflammation and haemorrhage of the small intestine (Heywood and Sortwell, 1979).

Organic arsenicals

The gastrointestinal tract appears to be the critical target of toxicity following oral exposure to MMA. Ingestion of 80 mg kg^{-1} of organic arsenicals causes vomitting, abdominal pain, hyperactive bowel and diarrhoea (Lee *et al.*, 1995).

A dose level of 72.4 mg MMA kg^{-1} per day led to a thickened wall, oedema and haemorrhagic, necrotic, ulcerated or perforated mucosa in the large intestine and a significant increase in the incidence of squamous metaplasia of the epithelial columnar absorptive cells in the colon and rectum. Squamous metaplasia was also observed in the colon of mice chronically exposed to 67 mg MMA kg^{-1} per day (Arnold *et al.*, 2003; Gur *et al.*, 1991).

Liver Diesease

Inorganic arsenicals

A number of studies revealed symptoms of hepatic injury after oral exposure of humans to inorganic arsenic. These effects were most frequently observed after repeated exposure to doses of 0.01–0.1 mg As kg^{-1} per day. Clinical examination confirmed liver damage (Liu *et al.*, 2002) and blood tests showed elevated levels of hepatic enzymes. Histological examination of the livers has revealed a consistent finding of portal tract fibrosis (Mazumder *et al.*, 2005). Individuals exposed more frequently to arsenic suffered from cirrhosis, which was considered to be a secondary effect of damage to the hepatic blood vessels.

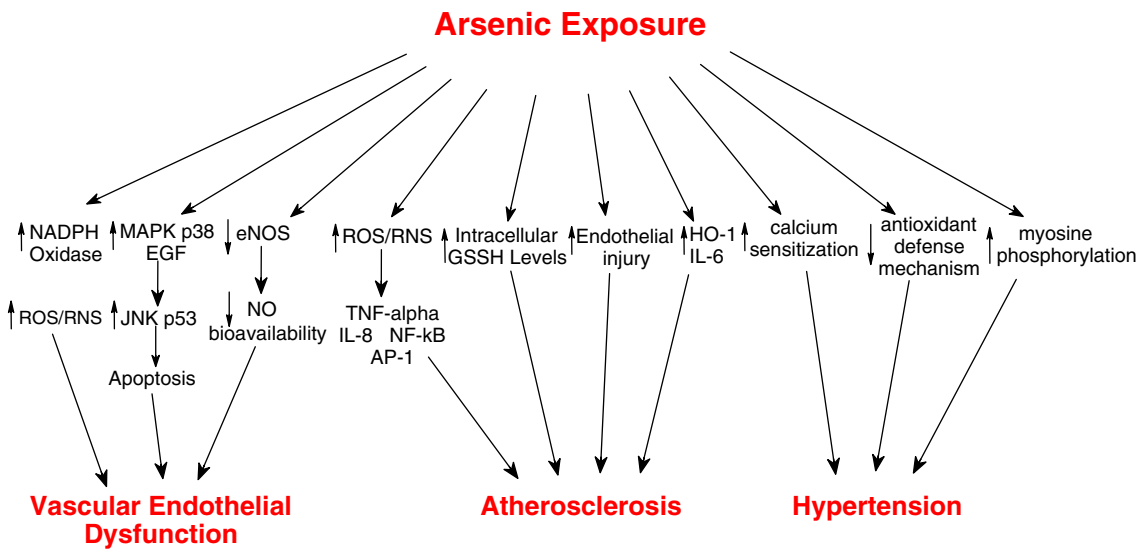

Figure 4. The influence of arsenic on the mechanisms of the vascular endothelial dysfunction, atherosclerosis and hypertension.

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Increased concentrations of zinc and copper were detected in the livers of rats receiving a single oral dose of 10 mg As kg^{-1} as sodium arsenite (Flora and Tripathi, 1998). Hepatic levels of malondialdehyde and glutathione were decreased in the livers of rats receiving 200 mg As kg^{-1} as GaAs (Flora *et al.*, 1998). An increase in peroxidation markers was reported in rats administered with 0.02 mg As kg^{-1} per day for 60 days from drinking water containing 2.5 mg sodium arsenite l^{-1} (Bashir *et al.*, 2006).

Organic arsenicals

No studies of the hepatic effects of organic arsenicals on humans have been reported. Histology of livers from rabbits repeatedly given MMA showed diffuse inflammation and mild hepatocellular degeneration (Jaghabir *et al.*, 1989). Rats exposed to a dose of 72.4 mg MMA kg^{-1} per day for 104 weeks showed a decrease in absolute liver weight. While rats exposed to DMA (Siewicki, 1981) did not exhibit any effect, mice exposed to oral doses of 720 mg DMA kg^{-1} exhibited decreased liver glutathione and cytochrome P-450 content and reduced serum ornithine decarboxylase activity (Ahmad *et al.*, 1999).

Renal Disease

Inorganic arsenicals do not cause any significant renal injury in humans. In some cases elevated levels of creatinine or bilirubin have been reported (Moore *et al.*, 1994). Similarly, animal studies indicated that the kidney is not a major target for inorganic arsenic. However, at high levels of exposure, mild histological changes in the renal tubules of monkeys have been noted. Animal studies have reported renal and urinary bladder effects following oral exposure to organic arsenicals. The urinary system is a more sensitive target for DMA than for MMA (Cohen *et al.*, 2001). Animal experiments have shown that DMA induced renal damage characterized by increased volume and pH of urine and decreased electrolyte levels, increased urinary calcium levels and an increase in water consumption. In addition, increased kidney weights and minimal tubular epithelial cell degeneration, tubular casts and focal mineralization were observed. Some studies on rats exposed to DMA have reported damage to the urinary bladder. The observed damage included altered bladder cell surfaces.

Neurological Disorders

Inorganic arsenicals

Inorganic arsenic can cause serious neurological effects, after both inhalation (Calderon *et al.*, 2001; Lagerkvist and Zetterlund, 1994) and oral exposure (Uede and Furukawa, 2003). This conclusion is based mainly on clinical observations and neurological examinations of exposed individuals.

Animal studies have shown that neurological effects following rat exposure to arsenic in the form of sodium arsenite involve changes in levels of neurotransmitters such as dopamine, norepinephrine and 5-hydroxytryptamine (Kannan *et al.*, 2001). Since adult animals appear to be much less susceptible to the neurological effects of inorganic arsenic than humans, studies in adult animals would probably not help to estimate a safe human exposure limit.

Recent findings indicate a possible association between arsenic in drinking water and neurobehavioral alterations in children (Tsai *et al.*, 2003). Adolescents from various regions of Taiwan and China exposed to low (0.0017–0.0018 mg As kg^{-1} per day) levels of inorganic arsenic in their drinking water showed a decreased performance in the switching attention task, while children in the high exposure group (0.0034–0.0042 mg As kg^{-1} per day) showed a decreased performance in both the switching attention task and in tests of pattern memory, relative to unexposed controls.

Ingestion of inorganic arsenic can cause injury to the nervous system. Exposure at a level of 2 mg As kg^{-1} per day or more can lead to encephalopathy, with symptoms of headache, mental confusion, seizures and coma (Bartolome *et al.*, 1999). Prolonged exposures to lower levels of arsenic (0.03–0.1 mg As kg^{-1} per day) are typically characterized by a symmetrical peripheral neuropathy (Chakraborti *et al.*, 2003; Foy *et al.*, 1992), which in its early stages is characterized by numbness in the hands and feet then further develops into a painful pins-and-needles sensation. Both sensory and motor nerves are affected and muscle weakness often develops (Goebel *et al.*, 1990). A certain degree of recovery is possible once the subject has been removed from the contaminated area, but most commonly this is only partial (Fincher and Koerker, 1987).

The most typical neurological feature of arsenic neurotoxicity is peripheral neuropathy which may last for several years (Mathew *et al.*, 2010). Studies on patients with As neuropathy have shown a reduced nerve conducting velocity in their peripheral nerves, and this has become a hallmark of As-induced neurotoxicity, as is is a typical feature of axonal degeneration. The majority of the unfavourable effects of Arsenic are caused by the inactivation of enzymes that are important for cellular energy metabolism, whereby As reacts with the thiol groups of proteins and enzymes and inhibits their catalytic activity. In a similar fashion to other neurodegenerative diseases, arsenic-induced neurotoxicity causes changes in cytoskeletal protein composition and hyperphosphorylation. These changes may lead to disorganization of the cytoskeletal structure, which is a potential cause of As-induced neurotoxicity.

Organic arsenicals

No neurological symptoms or brain lesions were observed following chronic exposure of rats to MMA (72.4 mg kg^{-1} per day) or mice to MMA kg^{-1} per day (67.1 mg; Arnold *et al.*, 2003). Two further studies in pigs indicated that oral doses of roxarsone can cause significant neurotoxicity in which the main feature is a time-dependent degeneration of myelin and axons (Kennedy *et al.*, 1986).

Reproductive Health Effects

Animal studies have shown that reproductive activity was unaffected in rats receiving doses of 8 mg of As_2O_3 from 14 days prior to mating. The evaluation of reproductive activity included a mating index, a fertility index and the precoital interval (time before mating) index (Holson *et al.*, 1999).

A more comprehensive, three-generation study of arsenic intake in drinking water in mice revealed a significant increase in the incidence of small litters and a trend toward a smaller number of pups per litter in all three generations of the treated group (Schroeder and Mitchener, 1971).

This conclusion was recently confirmed by another study which showed changes in several reproductive system end points, including reduced weights of the uterus and ovary and reduced ovarian and uterine peroxidase activities; inhibition of

steroidogenic enzymes and decreased estradiol levels relative to the controls (Chattopadhyay *et al.*, 2001, 2003).

Antioxidant Protection Against Arsenic Mutagenicity

Oxidative stress to DNA is recognized as an underpinning component of the mechanism of arsenic carcinogenesis (Valko *et al.*, 2005). Antioxidant enzymes are considered to be the first line of cellular defence against oxidative damage. Superoxide dismutase (SOD) and catalase (CAT) are the most important, first line antioxidant defence in cells exposed to oxygen. SOD catalyses the dismutation of superoxide into oxygen and hydrogen peroxide, while CAT catalyses the decomposition of hydrogen peroxide to water and oxygen. Arsenic-intoxicated rats revelaed reduced activity of SOD which was attributed to the enhanced production of superoxide radical anions.

A second line of cellular defence system against free radical-induced damage is provided by a thiol-based antioxidant system (Manna *et al.*, 2008). Decreased GSH pools and increased levels of lipid peroxidation due to arsenic toxicity were found to lead to a decrease in the activities of GST and GPx with a concomitant decrease in the activity of the GSH-regenerating enzyme GR.

A field trial was undertaken in West Bengal (a region whose population is exposed to high levels of arsenic in drinking water), to evaluate the role of the phytochemical, curcumin, from turmeric for its antioxidant and antimutagenic activity (Biswas *et al.*, 2010). Blood samples taken from volunteers in the region showed notable DNA damage and depleted antioxidant activity. However, following dosage with curcumin capsules for 3 months, the DNA damage was reduced, ROS generation and lipid peroxidation were suppressed, and the antioxidant activity of blood plasma was raised, thus offering the hope of some protective role for curcumin against DNA damage by arsenic.

The most effective known treatment for arsenic poisoning is chelation therapy; however such agents as British anti lewisite, sodium 2,3-dimercaptopropane-1-sulfonate, meso 2,3-dimercaptosuccinic acid etc. result in a number of undesirable side-effects (Flora *et al.*, 2007). It has been shown that supplementation of the chelating agent with antioxidants may be beneficial in achieving optimum effects.

Another study reported genotoxic effects of sodium arsenite (known for its genotoxic effects through ROS generation) in forming micronuclei in the polychromatic erythrocytes in the bone marrow cells of Wistar rats (Balakumar *et al.*, 2010). Supplementation by orally administered *α*-tocopherol (400 mg kg^{-1} of body weight) and ascorbic acid (200 mg kg^{-1} of body weight) to rats given 100 ppm of sodium arsenite in their drinking water for 30 days suggested a protective effect on the cellular antioxidant system and a modulation of arsenic-induced micronuclei formation.

CONCLUSION

Arsenic exposure affects millions of people worldwide. Epidemiological studies appear to provide an important guide for arsenic risk assessment in water, air or dust. Research work on arsenic poisoning has revealed that free radical-mediated oxidative damage is a common denominator of arsenic pathogenesis. A dose-dependent relationship between arsenic concentration and cancer incidence has been found, however, only among highly exposed populations.

Although arsenic-induced formation of various cancers has been widely studied, less attention has been paid to arsenic-induced cardiovascular disorders, even though epidemiological studies have shown that chronic arsenic exposure is associated with increased morbidity and mortality from cardiovascular disease. Arsenic has been found to initiate endothelial dysfunction by dinishing the integrity of vascular endothelium followed by inactivation of the eNOS, which therefore reduces the generation and bioavailability of nitric oxide and increases oxidative stress.

Arsenic-induced formation of ROS and subsequent depletion of antioxidant cell defences can result in disruption of the antioxidant/prooxidant equilibrium in mammalian tissues. Owing to its sulfydryl group binding capacity, arsenic can also inhibit the activities of many enzymes, especially those involved in the uptake of glucose in cells, fatty acid oxidation and production of glutathione.

Although the toxic and carcinogenic effects on humans exposed to arsenic have been well documented, the mechanisms by which arsenic induces health effects, including cancer, cardiovascular disorders, metabolic disease and other diseases are not well characterized. To provide a deeper understanding of the pathology of arsenic-induced diseases and the toxicology of arsenic in various organs, further research is necessary.

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