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Oxidative mechanism of arsenic toxicity and carcinogenesis

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Abstract

Arsenic is a known toxin and carcinogen that is present in industrial settings and in the environment. The mechanisms of disease initiation and progression are not fully understood. In the last a few years, there has been increasing evidence of the correlation between the generation of reactive oxygen species (ROS), DNA damage, tumor promotion, and arsenic exposure. This article summarizes the current literature on the arsenic mediated generation of ROS and reactive nitrogen species (RNS) in various biological systems. This article also discusses the role of ROS and RNS in arsenic-induced DNA damage and activation of oxidative sensitive gene expression. (Mol Cell Biochem **255**: 67–78, 2004)

Key words: arsenic, free radicals, oxidative stress, signal transduction, carcinogenesis

Abbreviations: A_L – human-hamster hybrid cells; BAEC – bovine aortic endothelial cells; CM-H_2DCFDA – 5′,6′-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate; DCFH – dichlorofluorescin; DMSA – meso 2,3-dimercaptosuccinic acid; DMA – dimethylarsinic acid; DMPO – 5,5-dimethyl-1-pyrroline N-oxide; EPR – electron paramagnetic resonance; GPx – glutathione peroxidase; H_2O_2 – hydrogen peroxide; HaCat – human keratinocyte cell line; $LOO^{\cdot}$ – lipid peroxyl radical; MDA – malondialdehyde; MMA – monomethylarsonic acid; NAC – N-acetyl-cysteine; NAME – nomega-nitro-L-arginine methyl ester; NO – nitric oxide; $O_2^{\cdot-}$ – superoxide anion; $^{\cdot}OH$ – hydroxyl radical; 8-OHdG – 8-Hydroxyl-2′-deoxyguanosine; ROS – reactive oxygen species; RNS – reactive nitrogen species; SOD – superoxide dismutase; PAEC – porcine aortic endothelial cells; PARP – poly (ADP-ribose) polymerase; TEMPOL-H – 4-hydroxy-2,2,6,6,-tetramethyl-1-hydroxypiperidine; VSMC – human vascular smooth muscle cells

Introduction

Arsenic is a naturally occurring element that is present in food, soil and water. The concentration of arsenite in drinking water varies in the range 0.01–3.7 mg/l (1.3–49 μM) [1]. There exist different types of arsenic in environment and human body, inorganic arsenic and organic arsenic [2–7]. Inorganic arsenic includes arsenite (As(III)) and arsenate (As(V)). The inorganic arsenics can be methylated to form monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) *in vivo* [7]. Although the process was previously considered a detoxification mechanism, recent studies *in vivo* indicate that methylated forms of arsenic may serve as co-carcinogens or tumor promoters [8–11]. Arsenate is a phosphate analog, which interferes with phosphorylation reactions and competes with phosphate in transport. Arsenite can react with the sulfhydryl groups of proteins and inhibits many biochemical pathways. Biologically, the trivalent arsenite is significantly more active than the pentavalent arsenate, including the ability to induce gene amplification in mammalian cells [12, 13], while arsenite and arsenate would have similar effects in vivo, as absorbed arsenate is mostly reduced to arsenite in blood [14, 15].

It has long been known that arsenic is toxic and carcinogenic. Epidemiological studies have indicated that people exposed to high levels of arsenic are prone to develop skin, bladder, liver, and lung cancers [16–25]. In addition to its carcinogenic effects, arsenic exposure has been suggested to

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play a role in black foot disease [26], type II diabetes mellitus [27], and cardiovascular diseases [28]. The mechanism of arsenic as a carcinogen and a toxin has been under intense investigation in recent years. Reactive oxygen and nitrogen species such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$), and nitric oxide (NO) are known to be important in mutagenesis and carcinogenesis [29, 30]. Many studies showed that free radicals were generated during arsenic metabolism in cells. Enhanced oxidative stress might be associated with the development of arsenic-related diseases including cancers. It has been demonstrated that reactive oxygen species (ROS) and reactive nitrogen species (RNS) are directly involved in oxidative damage to lipids, proteins and DNA in cells exposed to arsenic, which can lead to cell death. On the other hand, many recent studies have provided experimental evidence that arsenic can cause cell damage and death through ROS or RNS signaling pathways. This article summarizes the literature on the arsenic mediated generation of ROS and RNS in various biological systems and their roles in arsenic-induced cellular damage, and activation of oxidative sensitive signaling pathways.

Arsenic-mediated ROS and RNS generation and their detection

ROS refers to $O_2^{\cdot-}$, H_2O_2, $^{\cdot}OH$, singlet oxygen ($^1O_2$,), and peroxyl radical ($LOO^{\cdot}$) etc. Experimental results show that $O_2^{\cdot-}$ and H_2O_2 are produced in various cellular systems exposed to arsenite at different concentrations (Table 1). Arsenite generates detectable levels of $O_2^{\cdot-}$ in U937 cells at the concentration of 1–10 μM [31], human vascular smooth muscle cells (VSMC) at 7–16 μM [32], and human-hamster hybrid cells at 50 μM [33]. At environmentally relevant concentration or at non-lethal concentrations (below 5 μM), arsenic could also stimulates $O_2^{\cdot-}$ and H_2O_2 formation in vascular endothelial cells [34], as detected by electron paramagnetic resonance (EPR) spectrometry with spin trap agent 5,5-dimethyl-1-pyrroline N-oxide (DMPO). Moreover, the generation of $O_2^{\cdot-}$ and H_2O_2 has also been observed in human keratinocyte cell line (HaCat) with fluorescence and EPR techniques [Liu *et al.*, unpublished data]. The induction of H_2O_2 has been found in other type of cells exposed to arsenic. In HEL30 cells, arsenite at the concentrations of 1–50 μM elevated the levels of H_2O_2 as measured by the oxidation of DCFH [35]. The production of H_2O_2 appears to be involved in the induction of apoptosis by arsenite in NB4 cells [36] and CHO-K1 cells [37]. Hydrogen peroxide-resistant CHO cells are also cross-resistant to arsenite [38]. It has been suggested that arsenite-induced apoptosis in CHO-K1 cells was initiated by the production of $^{\cdot}OH$, which selectively activates protein kinase through *de novo* synthesis of macromolecules [37]. These results indicate that $O_2^{\cdot-}$ is likely the primary species induced by arsenic in various types of cells and the formation of $O_2^{\cdot-}$ leads to a cascade of other ROS such as H_2O_2 and $^{\cdot}OH$. In the presence of superoxide dismutase (SOD), $O_2^{\cdot-}$ dismutates to oxygen and H_2O_2 that is converted in a spontaneous reaction catalyzed by Fe^{2+} (Fenton reaction) to the highly reactive $^{\cdot}OH$ radical.

Table 1. ROS generation in various cellular systems induced by arsenic at different concentrations

Arsenic (μM)	ROS/ RNS	Cellular system	Method	Effective antioxidants/or inhibitor	References
< 5	$O_2^{\cdot-}$	PAEC	EPR-DMPO	SOD, DPI, apocynin	[34, 124]
< 5	H_2O_2	PAEC	Fluorescence-homovanillic acid		[34, 124]
> 5	H_2O_2	BAEC	Fluorescence	SOD	[40]
1–50	ROS	HEL30	Fluorescence-DCFH	Rotenone	[35]
50	$O_2^{\cdot-}$	U937	Fluorescence-DE	NAC, BAPTA-AM	[31]
50–300	$O_2^{\cdot-}$	HaCat	Fluorescence-DE	SOD, PBN	Liu *et al*. unpublished data
50–300	ROS	HaCat	EPR-DEPMPO	SOD, catalase	Liu *et al*. unpublished data
1–10	$O_2^{\cdot-}$	HVSMC	Chemiluminescence	SOD	[32]
7–16	ROS	A_L	Fluorescence-CM-H_2DCFDA	DMSO	[33]
7–16	ROS	A_L	EPR-TEMPOL-H	Catalase, SOD	[33]
1–2	H_2O_2	NB_4	Fluorescence-DCFH	Selenite	[36]
4–40	H_2O_2	CHO-K1	Fluorescence-DCFH	NAC, Trolox, Tempo	[37]
> 5	NO	BAEC	Fluorescence-nitrite		[40]
0–80	NO	CHO-K1	Fluorescence-nitrite	NAME	[53]

PAEC – porcine aortic endothelial cells; BAEC – bovine aortic endothelial cells; HVSMC – human vascular smooth muscle cells; A_L – human-hamster hybrid cells; CM-H_2DCFDA – 5′,6′-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate; TEMPOL-H – 4-hydroxy-2,2,6,6,-tetramethyl-1-hydroxypiperidine; DCFH – dichlorofluorescin; BAPTA-AM – bis-(*o*-aminophenoxy)ethane-N,N,N´,N´-tetraacetic acid acetoxymethyl ester; NAME – nomega-nitro-L-arginine methyl ester.

The above results demonstrate that arsenic exposure results in the generation of ROS in various cellular systems. However, the source or mechanism of the ROS formation remains unclear. It has been suggested that mitochondria may be sources of ROS production based on experimental findings. For example, the increase in cellular ROS induced by arsenite could be completely abrogated by rotenone in HEL 30 cells, indicating mitochondria as the intracellular source of ROS induced by arsenite [35]. Meanwhile, it has also been found that the ROS production was not affected by calcium ions [35]. It has been suggested that arsenite increases free radical production at the ubiquinone site of the respiratory chain and that mitochondria are the main source of ROS. Lynn *et al.* demonstrated that treatment with arsenite can increase intracellular $O_2^{\cdot-}$ levels and that this increase is probably due to activation of NADH oxidase because inhibition of the expression of NADH oxidase by transfecting a p22phox antisense oligonucleotide markedly reduced $O_2^{\cdot-}$ production in VSMCs [32]. However, others contended the conception that mitochondria were the main source of ROS induced by arsenic. Diphenyleneiodonium, an inhibitor of NAD(P)H oxidase and mitochondrial ROS production [39] showed no inhibitory effect on arsenite (20 μM)-induced $O_2^{\cdot-}$ generation in BAECs [40]. In addition to mitochondria, there are several other proposed sources of ROS. (a) Yamanaka *et al.* proposed the formation of intermediary arsine species [41–43]. By EPR analysis, a radical species believed to be $(CH_3)_2AsOO^{\cdot}$ was detected as a reaction product of dimethylarsine and molecular oxygen. This peroxyl radical, rather than active oxygen species, was assumed to play a major role in DNA damage [44]. Superoxide anion may also be produced during the process of $(CH_3)_2AsOO^{\cdot}$ generation [45]. (b) Methylated arsenic species can release redox-active iron from ferritin and have synergic effect with ascorbic acid to do so. Free iron plays a central role in generating harmful oxygen species by promoting the conversion of $O_2^{\cdot-}$ and H_2O_2 into the highly reactive $^{\cdot}OH$ radical through the Haber-Weiss reaction [46, 47]. (c) Another proposal is the oxidation of arsenite to arsenate [48]. Under physiological conditions, the oxidation process produces H_2O_2 as follows,

$$H_3AsO_3 + 2H_2O + O_2 \rightarrow H_3AsO_4 + H_2O_2 \quad \Delta G^0 = -40.82 \text{ kcal/mol}$$

Taken together, the available evidence shows a strong link between arsenic exposure and formation of ROS, but further studies are needed to sort out the exact sources of ROS.

Besides ROS, arsenic exposure can also affect the generation of RNS. NO is a messenger molecule that plays an important role in neurotransmission, vasodilation, and immune response. NO also possesses toxic effect such as prooxidant effect, genotoxicity and mutagenicity. Production of NO is mainly catalyzed by NO synthases, which consist of neuronal, endothelial, and inducible forms. There are controversial reports about NO production induced by arsenic. Pi *et al.* argued that prolonged exposure to arsenic impaired production of endothelial NO in human blood. The reported mean concentration of nitrite/nitrate in serum was 50% lower in arsenic-exposed human subjects, and the endothelial NO level was suppressed by 18% with 100 μM arsenite [49]. In porcine aortic endothelial cells, no increased NO production was observed under environmental relevant exposure level of arsenite [50]. De Vera reported that there was no increase in NO generation in hepatocytes and human liver cells induced by arsenite, which inhibited inducible NO synthase gene expression in cytokine-stimulated human liver cells and hepatocytes [51]. Arsenite also inhibited inducible NO synthase gene expression in rat pulmonary artery smooth muscle cells [52]. At levels of arsenite (< 5 μM) that did not change intracellular concentration of Ca^{2+}, no NO generation was detected directly by EPR [34, 53]. On the contrary, a 4-h treatment with arsenite above 5 μM induced a sharp increase in nitrite release in bovine aortic endothelial cells [40]. At greater than 20 μM of arsenite, Lynn *et al.* observed an arsenite concentration- and treating-time-dependent increase of nitrite level in the condition medium of arsenite-treated CHO-K1 cell cultures [33, 54]. The increase in nitrite levels suggests elevated NO production in these cells. Increased NO production also has been observed in C3H 10T ½ cells [55]. It appears that the stimulation of NO production by arsenite is through activation of eNOS by calcium. Nitric oxide seems to be involved in arsenite-induced DNA damage and pyrimidine excision inhibition [56]. These inconsistent discoveries indicate that the effect of arsenite on NO generation is likely cell type specific and arsenic concentration dependent.

Because of the technical difficulties associated with detecting ROS induced by arsenic, a variety of techniques have been utilized (as shown in Table 1), including chemiluminescence, fluorescence, and electron paramagnetic resonance (EPR). For EPR with spin trapping experiments, besides traditional spin trap DMPO [34], the hydroxylamine TEMPOL-H was used as a probe to detect generation of ROS in cells with EPR. TEMPOL-H readily penetrates plasma membranes and detects free radicals, particularly $^{\cdot}OH$ and $O_2^{\cdot-}$ with high sensitivity and specificity [33, 54]. In reaction with ROS, TEMPOL-H is converted to Tempol, a nitroxide, which is EPR active and more stable than DMPO spin adduct [57]. The use of dichlorofluorescein to measure ROS generation in cells has provided a means to quantify the relative amounts of ROS generation. CM-H_2DCFDA is considered an improved version of the original dye that tends to leak out of cells with time, which provides a better retention in live cells and more reliable fluorescent signals. The measurement of NO has been carried out by EPR-spin trapping technique, indirect detection of nitrite level, or assessment of NOS activity. In addition to the direct or indirect measurement of ROS and RNS, effect of radical scavengers and enzyme inhibitors on arsen-

ite induced cellular damage provided further evidence of the involvement of free radicals in the toxicity and carcinogenesis of arsenite, as discussed in the following section.

In addition to the direct detection of ROS or their metabolites, an increase in the rate of oxygen consumption in arsenite-treated cells would also imply that there is increased metabolism and/or ROS formation. A significant increase in oxygen consumption has been observed in endothelial cells in the presence of arsenite at 5 μM with EPR oximetry [34]. In suspensions of tubules from the rabbit kidney, arsenate increased oxygen consumption rates by 20.5% and decreased NADH fluorescence by 10.8%. These effects on metabolism were concentration dependent and magnified in the presence of ouabain, which specifically binds to and inhibits the sodium-potassium ATPase. The data indicate that arsenate's main effect is to uncouple oxidative phosphorylation [58].

Effect of antioxidants and radical scavengers

Antioxidants are substances that have the ability to inhibit free radical generation, scavenge free radical, and/or reduce the oxidation and damage caused by these radicals. The antioxidant defense system is composed of several lines as outlined by Noguchi and Niki [59]. Generally, the antioxidant system includes enzymes such as SOD, catalase, and glutathione peroxidase (GPx) etc., free radical scavengers, inhibitors to free radical generating enzymes, and metal chelators. The use of different antioxidants has been found beneficial in various cellular systems exposed to arsenic. The addition of catalase or GPx to cultures reduced the arsenite-induced micronuclei in xrs-5 cells. Simultaneous treatment with mercaptosuccinate, an inhibitor of GPx, and 3-aminotriazole, an inhibitor of catalase, synergistically increased the arsenite-induced micronuclei [60]. SOD and catalase had a protective effect against arsenic-induced DNA damage [61]. Arsenite induced ROS generation was reduced by SOD, 4,5-dihydroxy-1,2-benzenedisulfonic acid disodium salt, and diphenylene iodinium [32]. Catalase effectively reduced the frequency of arsenite-induced micronuclei [62]. Arsenic-induced DNA strand breaks could be decreased by NO synthase inhibitors, O_2^- scavengers, and peroxynitrite scavengers and increased by superoxide generators and NO generators [40]. S-methyl-L-thiocitrulline and Nω-nitro-L-arginine methyl ester, inhibitors of nitric oxide synthase, could suppress the arsenite-induced NAD depletion, DNA strand breaks, micronuclei and poly (adenosine diphosphate rebosylation) [53]. Simultaneous treatment with arsenite plus antioxidant (Trolox and Tempo), copper ion chelator (neocuproine), protein kinase inhibitor (H-7), or protein synthesis inhibitor (cycloheximide) reduced the fraction of sub-G1 cell and internucleosomal DNA degradation induced by arsenite [37]. Trolox, neocuproine, or cycloheximide given after arsenite treatment also effectively reduced apoptosis [37]. Furthermore, the enhanced DCF fluorescence by sodium arsenite exposure in human fibroblasts was inhibited by butylated hydroxytoluene. Sodium arsenite-enhanced heme oxygenase synthesis was inhibited by co-treatment of cells with the antioxidants sodium azide and dimethyl sulfoxide [63].

In addition to the antioxidant described above, it is noteworthy to mention the role of N-acetyl-cysteine (NAC). N-acetyl-cysteine, a thiol-containing antioxidant, seems to be a potential antioxidant in inhibiting cellular damage caused by arsenite. In contrast to partial protection by the caspase-3 inhibitor, NAC provided marked protection from arsenite-induced apoptosis and eliminated the activation of p38, JNK, and caspase-3, and the generation of ROS [64]. NAC significantly inhibited arsenite-induced apoptosis and intracellular fluorescence intensity [65]. NAC blocked the suppression of FasL expression in response to arsenite, which suggested that arsenite initiates endothelium dysfunction, at least partly, by suppressing the FasL expression through activating ROS sensitive endothelial cell signaling [66]. NAC reduced the fraction of sub-G1 cell and internucleosomal DNA degradation [37]. Moreover, studies using animal model showed that treatment with NAC and meso 2,3-dimercaptosuccinic acid (DMSA) alone partially reversed arsenic-induced reduction in hepatic GSH and malondialdehyde (MDA) increases in hepatic, brain and red blood cells. Treatment with DMSA also produced an effective depletion of blood and hepatic arsenic concentrations. In the liver, most of these parameters were more effectively reversed by combined treatment with NAC and DMSA compared with the effects of either drug alone [67].

Clearly, enzyme antioxidants (SOD, catalase and GPx), ROS scavengers, metal chelators and NOS inhibitors protect cells from oxidative damage induced by arsenic. These observations again provide evidence that ROS and RNS are generated in biological systems exposed to arsenic and that these reactive species play an important role in arsenic-mediated toxicity and carcinogenecity. NAC is regarded not as a direct antioxidant, but it increases the availability of GSH in cells. GSH plays a critical role in maintaining cellular redox homeostasis. Arsenic has the ability to complex with -SH groups, thus depleting cellular GSH levels and thiol status. The efficient effect of NAC indicates that arsenic changes cellular redox homeostasis by binding with GSH, which is its natural property. These observations suggest that the toxicity of arsenite could be carried out not only through the generation of ROS and RNS, but also by directly attacking SH groups. It also implies that the use of proper antioxidants can eliminate or reduce cellular damages caused by arsenic.

Biomarkers for oxidative stress induced by arsenic exposure

The production of ROS and RNS by arsenic usually results in oxidative stress in cells. Oxidative stress has been involved in mediating many deleterious effects of arsenic. A variety of biomarkers for oxidative stress induced by arsenic exposure have been determined, including DNA damage, lipid peroxidation, redox enzyme activity, and decreased antioxidant defense levels.

DNA damage

Increasing evidence shows that arsenic is genetoxic. Arsenic exposure leads to various types of DNA damage. This carcinogen has been reported to promote chromosomal aberrations, sister chromatid exchange, and induce DNA hyper and hypomethylation [13, 68]. Arsenic interferes with DNA methyltransferases, resulting in inactivation of tumor suppressor genes through DNA hypermethylation. Arsenite increased DNA migration as well as the percentage of cells with tails in MRC-5 cells [69]. The decrease in the rate of DNA migration could be a consequence of the induction of DNA cross-links by organic arsenicals. Other studies suggest that arsenic-induced malignant transformation is linked to DNA hypomethylation subsequent to depletion of s-adenosyl-methionine, which results in aberrant gene activation, including oncogenes [70]. It has also been proposed that genetic damage induced by exposure to arsenic may be due in part to inhibition of DNA repair [71–75]. DNA damage caused by arsenic has especially been observed in other two forms, DNA strand break and DNA oxidation.

Arsenite-induced DNA strand breaks were observed by alkaline single-cell gel electrophoresis in human fibroblast cells [76]. Treatment with arsenite increased DNA strand breaks in VSMCS [32]. It has been reported that a high concentration of arsenic is required to induce DNA strand breaks [74, 77, 78]. Arsenic-induced strand breaks were detected in a concentration-dependent manner in murine keratinocyte line JB6. With extended arsenic exposure, significant levels of DNA strand breaks were apparent at arsenic concentrations below 5 μM [Liu *et al.*, unpublished data]. However, the levels of DNA strand breaks could be under-estimated by the method used in the studies. Wang *et al.* argued that the level of detectable DNA strand breaks is low at any given time point because DNA strand breaks appear only temporarily during excision repair. They demonstrated that arsenite at 0.25 μM induced DNA strand breaks in both human leukemia cells and Chinese hamster ovary cells, and formamidopyrimidine-DNA glycosylase and proteinase K greatly increased DNA strand breaks in arsenite-treated cells. These observations indicate that a large portion of arsenite-induced DNA strand breaks come from excision of oxidative DNA adducts and DNA-protein cross-links [79], confirming that arsenite is able to induce a high level of oxidative DNA adduct and DNA protein cross-links as reported [32, 80, 81]. DNA stand breaks may be caused by ROS induced by arsenic. Nitric oxide synthase inhibitors, Nω-nitro-L-arginine methyl ester and s-methyl-L-thiocitrulline, had no apparent effects on arsenite-induced DNA strand breaks. However, SOD, catalase, diphenylene iodinium, DMSO, d-mannitol, and pyruvate effectively reduced arsenite-induced DNA strand breaks. The results suggest that ROS, but not nitric oxide, are involved in arsenite induced DNA strand breaks [32, Liu *et al.*, unpublished data]. DNA single strand breakage is an obligatory trigger for the activation of poly (ADP-ribose) polymerase (PARP), which can result in consumption of NAD, the depletion of ATP and cell death. PARP also play a role in DNA replication, DNA repair, gene expression and carcinogenesis [82]. DNA strand breaks can also cause chromosomal rearrangements.

8-Hydroxyl-2′-deoxyguanosine (8-OHdG) is considered one of the major products of ROS induced DNA damage and a biomarker of oxidative stress to DNA. Increased level of 8-OHdG has been observed in cellular, animal models and human tissues exposed to arsenic. Wanibuchi *et al*. observed that formation of 8-OHdG was significantly increased in male F344 rat liver after administration of DMA during 8 weeks [83]. In another animal model, Yamanaka *et al.* observed a significant increase in urinary 8-OHdG levels induced by DMA at 3–9 h [84]. In addition, increase of 8-OHdG has been found in human tissues resulted from exposure to arsenic. Matsui *et al.* studied the presence of 8-OHdG by immunohistochemistry using N45.1 monoclonal antibody in 28 cases of arsenic-related skin neoplasms and arsenic keratosis as well as in 11 cases of arsenic-unrelated Bowen's diseases. The frequency of 8-OHdG positive cases was significantly higher in arsenic-related skin neoplasms (22 of 28; 78%) than in arsenic-unrelated Bowen's disease (one of 11; 9%) ($p < 0.001$). Furthermore, 8-OHdG was also detected in normal tissue adjacent to the arsenic-related Bowen's disease lesions [85].

Lipid peroxidation

Exposure to arsenite results in lipid peroxidation. It was reported that lipid peroxidation was elevated in house flies exposed to arsenic by up to 2.9-fold [86]. Lipid peroxidation was increased one hour after the arsenic exposure (15.86 mg/kg body weight) in liver from male Wistar rats [87]. Twelve weeks of arsenic exposure increase oxidized glutathione and promote MDA production in both liver and brain samples. In addition to a significant reduction in delta-aminolevulinic

acid dehydratase activity and GSH levels, a marked elevation in MDA production may also contribute to arsenic-induced oxidative stress [67]. A long term study showed that lipid peroxidation was increased significantly after 6 months of arsenic feeding (3.2 mg/l) in male BALB/c mice [88]. Oral administration of orpiment (50 mg/kg body wt) caused an increase in the lipid peroxidation (78%) [89]. However, when adult male rats were treated orally with sodium arsenate (10 mg As/kg/day) for 2 days, there was no increase in the level of lipid peroxidation as measured by the formation of thiobarbituric acid-reacting substances in liver, but decreased plasma level of uric acid and increased the plasma triglycerides content without modifying vitamin E levels. Both total lipoproteins and low-density lipoprotein plus low-density lipoprotein fractions demonstrated greater propensity for *in vitro* oxidation than the corresponding untreated rats [90].

Reduced antioxidant defense level

Oxidative stress results from imbalance between free radical generation and antioxidant defense system. There is reported evidence that exposure to arsenic reduce antioxidant levels. Decreased antioxidant levels in plasma from individuals exposed to arsenic in Taiwan have been reported by Wu *et al.* [91]. There was a significant inverse correlation of plasma antioxidant capacity with arsenic concentration in whole blood. A significant reverse dose-response relationship with arsenic-related ischemic heart disease was also observed for serum level of α- and β-carotene [92].

GSH is very important in maintaining cellular redox status. Depletion of GSH is considered a marker of oxidative stress [93]. Glutathione plays a very important role in changes of cellular redox status induced by arsenic. There are three ways that arsenic can reduce cellular GSH levels. (a) GSH is proposed as the electron donor for the conversion of pentavalent to trivalent arsenicals [94, 95]. (b) Arsenite has high affinity to GSH. (c) Free radicals induced by arsenic can oxidize GSH. Therefore, exposure to arsenite is likely to cause depletion of GSH level. A high environmental level arsenite has been associated with lower GSH antioxidant protection [96]. Maiti *et al.* reported that one hour after the arsenic exposure (15.86 mg/kg body wt), GSH concentration was significantly decreased in the liver of male Wistar rats [87]. Oral administration of orpiment (50 mg/kg body wt) caused significant decreases GSH level (47%) in rat liver microsomes [89]. After 6 months of arsenic feeding (3.2 mg/l), hepatic GSH and the enzymes glucose-6-phosphate dehydrogenase and GPx were significantly lowered in male BALB/c mice. Twelve weeks of arsenic exposure was found to deplete GSH levels [67]. However, GSH level in the liver of mice increased after exposure to arsenic for 2 months while prolonged exposure to arsenic decreased GSH level [97]. Other reports also show that nonlethal arsenic concentrations elevate intracellular GSH levels [98, 99]. These results argue that effect of arsenic on GSH level is dependent on arsenic concentration and cellular response to arsenic attacks.

Enzyme activity as a marker for oxidative stress

SOD, catalase and GPx are three major enzymes that metabolize ROS. Effects of arsenic on the activity of the three enzymes have been reported in various systems, but the results are mixed. Increased activity of these enzymes by arsenic has been observed by several research groups. Both catalase and SOD activities increased in response to low concentrations (0.01-0.1 μM) of arsenic in developing maize embryos and to a wider range of arsenic concentrations (0.01–10 μM) in germinating embryos [100]. The levels of SOD, glutathione transferase, and glutathione reductase increased 1.3, 1.6, and 1.5 times, respectively in insects induced by arsenite [86]. Acute arsenic exposure significantly increased the GPx activity in liver of rat [87]. Oral administration of DMA increased the activities of mitochondrial SOD and GPx in mouse lung at 6 h or longer after dosing, while cytosolic SOD and catalase did not [43]. On the other hand, reduced activity of the enzymes by arsenic has also been reported. Hepatic catalase activity was significantly reduced at 9 months in the arsenic-fed group, while glutathione-S-transferase and glutathione reductase activities were also significantly reduced at 12 and 15 months [88]. Sodium arsenite treatment resulted in slightly decreased GPx activity and significantly decreased catalase activity in human fibroblasts [63].

Arsenic exposure also has an effect on other enzymes. Thioredoxin reductase is an NADPH-dependent flavoenzyme that catalyzes the reduction of many disulfide-containing substrates and plays an important role in the cellular response to oxidative stress. Arsenite and especially methylarsine (III) caused a concentration-dependent reduction in thioredoxin reductase activity in cultured rat hepatocytes [101]. The inhibition of thioredoxin reductase activity has been associated with altered cell dynamics and the induction of apoptosis [102–104]. It is possible that arsenic induced apoptosis is mediated through the loss of thioredoxin reductase activity. Furthermore, heme oxygenase, an oxidative stress protein, and peroxidase could also be induced by arsenite in various human cell lines [98, 99].

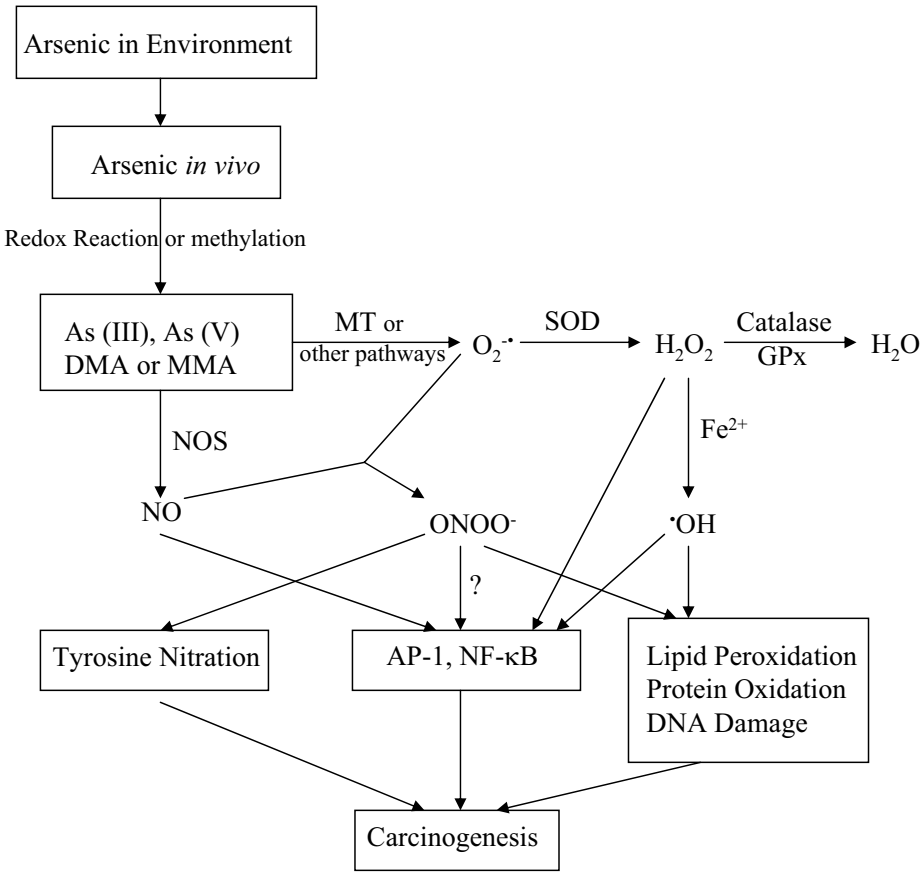
Arsenic-mediated signal transduction pathways associated with oxidative stress

Arsenic induces apoptosis and carcinogenesis, both of which are associated with cellular signaling pathways. ROS signaling

is critical for the responses of cytokines, growth factors, and activation or inactivation of transcription factors that promote carcinogenesis [105–107]. Arsenic shares many properties of tumor promoters by affecting specific cell signal transduction pathways involved in cell proliferation. It has been reported that signal transduction pathways are involved in the responses of several cell lines to arsenite. For example, arsenic may stimulate keratinocyte-derived growth factors [108] and induce overproduciton of interleukin-1α in murine keratinocytes [35], which may play a part in the initiation of cutaneous inflammatory hyperplastic diseases. Sub-lethal doses of arsenite resulted in activation of all three major mitogen-activated protein kinases pathways, ERK, JNK and p38 [109]. Both AP-1 and NF-κB are considered stress response transcription factors that govern the expression of a variety of pro-inflammatory and cytotoxic genes [110]. p53 gene is an important tumor-suppressor gene whose protein product plays an important role in cell cycle control, apoptosis, and control of DNA repair. Effects of arsenic on NF-κB, AP-1 and p53 activation are discussed as follows.

AP-1 and NF-κB

AP-1 is a heterodimer of the protein products of FOS and JUN immediate-early response gene family that controls the expression of many genes. NF-κB is involved in a wide variety of biological responses. In particular, it is implicated in inflammatory reactions, growth control and apoptosis, and is the first eukaryotic transcription factor shown to respond directly to oxidative stress in certain types of cells. Both NF-κB and AP-1 have been affected in various cells exposed to arsenic. Arsenite can activate AP-1 and NF-κB in BEAS-2B cells [111]. Arsenic induced a concentration- and time-dependent increase in cellular oxidative activity, which was followed by activation of redox-sensitive transcription factors such as NF-κB and AP-1 in HEL30 cells [35]. Arsenate at doses of 50–250 μM produced a complex profile of activity showing a significant dose-dependent induction of the NF-κBRE promoter in recombinant HepG2 cell lines [112]. Arsenic significantly altered nuclear binding levels of AP-1 and NF-κB to their respective cis-acting elements in human



Scheme 1. Mechanism of arsenic toxicity and carcinogenesis.

MDA-MB-435 breast cancer and rat H4IIE hepatoma cells [113]. Gel-shift assays indicated that 10 μM arsenite resulted in an enhanced DNA-binding activity of both AP-1 and NF-κB [114]. Within 1 h following addition of non-cytotoxic concentrations (< 5 μM) of arsenite in vascular endothelial cells, there was increased nuclear retention of NF-κB binding proteins. Supershift analysis demonstrated that p65/p50 heterodimers accounted for the majority of proteins binding consensus κB sequences in cells treated with arsenite [98]. Mice exposed to 0.01% sodium arsenite in drinking water developed hyperplasia of the bladder urothelium within 4 weeks of exposure, accompanied by a persistent increase in DNA binding of the AP-1 transcription factor [115]. Arsenate produced marked activation of the AP-1 transcription complex and increased NF-κB p65 protein level in mice liver [116].

p53

p53 plays a guarding role in maintaining genome integrity and accuracy of chromosome segregation. The effects of arsenic on p53 activation has been emphasized in recent years. Under acute exposure scenarios, significant induction of growth inhibitory proteins p53 and p21 was observed in different cell types following arsenite treatment. Immunoblots showed an increased expression of p53 gene with 1 μM sodium arsenite in Jurkat cells and 10 μM sodium arsenite in HeLa and a lymphoblast cell line transformed with Epstein-Barr virus cells [117]. Long-term (14 day) low dose (0.1 μM) arsenite caused a modest increase in p53 expression in WI38 normal human fibroblasts, while only toxic (50 μM) concentrations increased p53 levels after short-term (18 h) exposure [118]. Accompanying the appearance of DNA strand breaks was a significant accumulation of p53 in arsenite-treated human fibroblast cells, as demonstrated by immunoblotting and immunofluorescence techniques. p53 downstream proteins, such as p21 and the human homologue of murine double minute-2, were also significantly induced by arsenite treatment [76]. Arsenate at high concentrations (50–250 μM) produced a complex profile of activity showing a significant dose-dependent induction of the p53RE promoter in recombinant HepG2 cell lines [112]. Arsenite could up-regulate p53 protein in human gastric cancer MGC-803 cells [119]. In addition, arsenite has been reported as a novel potential therapeutic agent for acute promyelocytic leukemia. The mechanism involves arsenite induced apoptosis and G(1) phase accumulation by enhancement of p53 [120]. Although increasing studies suggest that arsenite induces p53, there was a study showing that after HaCat was exposed to environmentally relevant concentrations (< 1 μM) of arsenite for 14 days, arsenite decreased its p53 levels with a concomitant increase in the p53 regulatory protein mdm2 levels in a dose- and time-dependent manner. The disruption of the p53-mdm2 loop regulating cell cycle arrest may be a model for arsenic-related skin carcinogenesis, specially in tissue with elevated mdm2 levels [121].

Various studies have proved that ROS are involved in the activation of redox-sensitive transcription factors. ROS may act as a 'fast-acting third messenger molecules' in inducing the activation of redox-sensitive transcription factors such as NF-κB and AP-1. Transcription factors AP-1 and NF-κB have been implicated in the inducible expression of a variety of genes in response to oxidative stress. Activation of p53 is also considered a cellular response to oxidative stress. As discussed above, arsenic induces oxidative stress in many cellular systems. Arsenic mediated signal transduction pathways are likely associated with oxidative stress. Indeed, the activation of NF-κB and AP-1 and the production of IL-1α are followed by ROS production, and substance inhibiting ROS production from mitochondria decreases the activation and production [35]. H_2O_2 is apparently involved in the induction of apoptosis by arsenite in NB4 cells [36] and CHO-K1 cells [37]. H_2O_2 might play a role as a mediator to induce apoptosis through release of cytochrome c to cytosol, activation of CPP32 protease, and PARP degradation [65]. Nitric oxide is believed to be involved in the induction of poly(ADP-ribosylation) by arsenic [53], which is known to be involved in oxidant-induced cellular response [122]. In addition to oxidative stress, there may be other mechanisms by which arsenic affects signal transduction pathway. Arsenite may inhibit NF-κB activation through reaction with a critical cysteine in the activation loop of IκB kinase [123].

Summary

Based on the numerous experimental results over the past decades, particularly in the last few years, it is clear that oxidative stress plays an important role in the molecular mechanism of arsenic-induced toxicity and carcinogenesis. Exposure to arsenic will generate NO and $O_2^{\cdot-}$ that is subsequently converted to other more damaging reactive species such as $^{\cdot}OH$ radical and $ONOO^-$. The reaction and interaction of these reactive species with target molecules lead to oxidative stress, lipid peroxidation, DNA damage, and activation of signaling cascades associated with tumor promotion and/or progression (Scheme 1). Antioxidants that can inhibit, reduce, or scavenge the production of ROS and RNS induced by arsenic can not only decrease direct cellular damage such as lipid peroxidation, enzyme inactivation and DNA oxidation caused by arsenic, but also ameliorate cell injuries or death by redox signaling pathways activated by arsenic exposure. Further understanding of the mechanisms of free radical generation and the targets of these redox signaling induced by arsenic will provide potential therapeutic intervention in arsenic toxicity and carcinogenesis.

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