Current Topics in
Toxicology
Vol. 10, 2014

Review

Update on the oxidative stress associated with arsenic exposure

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ABSTRACT

Even though oxidative stress is currently widely accepted as a factor in As toxicity in both the liver and kidney, the mechanisms are not yet clearly understood. It has been suggested that As may induce oxidative stress by cycling between oxidation states of metals such as Fe, or by interacting with antioxidants and increasing inflammation, resulting in the accumulation of free radicals in cells. Major As-induced reactive oxygen species (ROS) include superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and peroxyl radicals. On the other hand, molecular oxygen reacts with dimethylarsine (a trivalent As form and a minor *in vivo* metabolite of dimethylarsinic acid) to form dimethylarsinic radical and $O_2^{\bullet-}$. Further, the addition of another molecule of molecular oxygen may result in a dimethylarsinic peroxyl radical and these As radicals are known to be detrimental to cells. It has been shown that As induced free radical formation in mouse livers. Experimental results have shown the generation of $O_2^{\bullet-}$ and H_2O_2 after As exposure in some cell lines such as human vascular smooth muscle cells, human-hamster hybrid cells, and vascular endothelial cells. Whereas, induction of H_2O_2 have been shown in other cell lines such as HEL30, NB4, and CHOK1 after exposure to As. The objective of the present review is to summarize the current knowledge on the role of oxidative stress in As toxicity in brain.

KEYWORDS: reactive oxygen species (ROS), arsenic (As), human health, brain

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1. INTRODUCTION

Reactive oxygen species (ROS) are highly reactive oxidants/reductants and are mainly regarded as hazardous species whose production in cellular and extracellular systems has to be tightly controlled by antioxidants and radical scavenging biochemical reactions. However, the importance of radical species in cellular signaling and in the maintenance of homeostatic conditions has been recognized. Some radicals such as the very short-lived and extremely hazardous hydroxyl radical ($^{\bullet}OH$) are still regarded as highly reactive and dangerous, but many other more stable species have been postulated as signaling molecules in cellular growth, or as oxidants that assure an appropriate oxidation state of cellular compartments and the biochemical structures, and elements they contain [1]. According to the present view, a basal amount of ROS is formed at all times in all aerobic cells, and the steady state concentration of ROS in each cell or compartment depends on the formation rate of the radical, its reactivity and the concentration of available reaction partners. Several decades ago, Chance *et al.* [2] calculated the steady state concentration of $^{\bullet}OH$, based on measurements of superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) production rates in rat liver cells assessed

using biochemical techniques. More recently, Antunes *et al*. [3] included nitric oxide (NO) into the picture of radical production and consumption by rat tissues. Lately, by applying electronic paramagnetic resonance (EPR) spectroscopy techniques, Galleano *et al*. [4] and Galatro *et al*. [5] developed simple kinetic studies to estimate ascorbyl radical ($A^{\bullet}$) steady state concentration in rat plasma and photosynthetic cells, respectively. There is a need for finding accurate methods to assess oxidative stress; however it seems very unlikely that any universal indicator of such a kind could be found easily. There is an increasing interest in the use of $A^{\bullet}$ content in biological tissues as an informative, non-invasive and natural indicator of oxidative stress [6] in the hydrophilic medium. It has been suggested that the steady state concentration of $A^{\bullet}$ in tissues may be considered as a useful but not a universal indicator characterizing oxidative stress intensity, since ascorbate (AH^{-}) oxidation rate depends on AH^{-} concentration, pH and non-disproportionation mechanisms of $A^{\bullet}$ decay. Recently, data obtained from different systems suggested that the quality of the indicator improves if AH^{-} content is also considered when it is affected by oxidative conditions. This factor was taken into account and the ratio $A^{\bullet}/AH^{-}$ content is used as a general indicator of oxidative stress in the hydrophilic medium.

Arsenic (As) exposure is believed to generate oxidative stress, indicated as an increase in the steady state concentration of oxygen radical intermediates. The main aspects of cellular metabolism of As are briefly reviewed here. Special emphasis on the role of As in the oxidative damage to lipid membranes is made by using both, *in vitro* and *in vivo* models [7-12]. Finally, the impact of As on human health is also highlighted. Taken as a whole, it is important to point out that from a pathological and toxicological viewpoint, further studies are required to assess ways to limit As-induced damage, by minimizing the formation and release of free radicals in tissues, when cellular As steady state concentration is increased.

2. Main features of As biochemistry

As is a metalloid element, and its environmental behavior and metabolism are similar to the physicochemical characteristics of both metal and non-metal elements. As occurs mainly as arsenate (iAs^{V}), or as arsenite (iAs^{III}), when anaerobic conditions are present in drinking water [13]. As is mainly metabolized in liver by repetitive reduction and oxidative methylation, and these pathways are primarily mediated by arsenic methyltransferase (AS3MT) (+3 oxidation state) [14]; however, an independent minor AS3MT methylation pathway has also been described [15]. Till now, the specific mechanism of As methylation *in vivo* has not been fully understood and remains under debate.

In human urine, the major metabolites of inorganic arsenicals such as arsenite (iAs^{III}) and arsenate (iAs^{V}) are monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}) [16]. Human recombinant AS3MT catalyses the transfer of a methyl group from S adenosyl-L-methionine to As and produces monomethyl- and dimethyl-arsenicals only when As triglutathione [$As^{III}(SG)_3$] or monomethylarsonic diglutathione ($MMDG^{III}$) are present in the reaction mixture [17]. Both $MMDG^{III}$ and dimethylarsinic glutathione ($DMAG^{III}$) are unstable compounds in solution when the reduced glutathione (GSH) concentration is lower than 1 mM, and are hydrolyzed and oxidized to MMA^{V} and DMA^{V}, respectively [17]. The intermediate arsenicals monomethylarsonous acid (MMA^{III}) and dimethylarsinic acid (DMA^{III}) are highly toxic compared to the pentavalent forms [8], and are considered as the forms that are mostly responsible for As toxicity, including carcinogenesis [13]. On the other hand, glutathione S-transferase-omega (GSTO) may play an important role in the reduction of pentavalent arsenicals and the subsequent formation of As-glutathione conjugates [18, 19]. Additionally, the conjugation of iAs^{III} with GSH has been shown to be able to proceed even in the absence of these enzymes when a hepatic level of GSH > 10 mM [20] is present within the reaction mixture [17]. Moreover, different individuals exhibited different urinary As metabolism patterns even after exposure to the same amount of As in the environment [21]. Gender and ethnicity affect these differences and glutathione S-transferase omega 1 (GSTO-1) and/or AS3MT polymorphisms could be effectors too [21, 22].

Yamanaka *et al*. [23-26] and Mizoi *et al*. [27] identified, from the metabolism of DMA, the formation of reactive compounds implicated in

DNA strand break. These products were also associated with the initiation and promotion of both skin and lung cancers. The metabolic pathways proposed included: (i) formation of a dimethylated As peroxide, an oxidative stress promotor from DMA, and (ii) formation of dimethylarsenic [$(CH_3)_2As^{\bullet}$] and dimethylarsenic peroxy radicals [$(CH_3)_2AsOO^{\bullet}$]. These sequences of events are likely to occur when $DMAG^{III}$ is reduced to a dimethylarsine by the action of a GSH reductase and NADPH. Finally, this product may react with molecular oxygen to form dimethylarsine radical. In the presence of high oxygen pressure, as in the lung environment, the dimethylarsine radical may react with molecular oxygen leading to the formation of dimethylarsine peroxyl radical. The diagram shown in Figure 1 briefly summarizes the described transformations.

3. Models employed to study oxidative stress associated with As toxicity

Table 1 briefly summarizes some of the reported protocols recently published. As indicated there, the main assays tested include oxidation of 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA, general measurement of reactive species), thiobarbituric acid-reactive substances (TBARS, damage to lipids), content of carbonyl proteins (oxidation of proteins), 8-hydroxydeoxyguanosine (8-OHdG, damage to DNA) and antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT, etc.). The effect of As exposure was studied in several tissues, such as liver, blood, kidney, spleen, and only a few of them included the brain. Relevant experimental results have shown the generation of $O_2^{\bullet-}$ and H_2O_2 after As exposure in some cell lines such as human vascular smooth muscle cells, human-hamster hybrid cells, and vascular endothelial cells, whereas other cell lines such as HEL30 [28], NB4 [29], and CHOK1 [30] have shown to induce H_2O_2.

However, it is very difficult to establish comparisons among these studies, mostly due to the variety of the protocols employed. It is important to point out that no recent publications on the role of oxidative stress in As toxicology included experiments employing direct assays to detect the oxidative or nitrosative species generation *in vivo*. To gain a deeper understanding of the pathology of As-induced diseases and the toxicology of As in various organs, further research is necessary.

4. As and oxidative stress in brain

As interacts with thiol groups; thus, it can be toxic by directly blocking essential sulfhydryl groups of proteins and enzymes [31]. This kind of binding can perturb the function of enzymes of carbohydrate metabolism, such as succinic and pyruvate dehydrogenases [32]. Also, its binding to free sulfhydryl groups of membrane proteins induces a marked decrease of free sulfhydryl groups and may alter the intracellular signaling mechanisms [33]. Arsenite inhibits glutathione reductase (GR) and diminishes the intracellular level of GSH, which is important in cellular redox balance and protection against oxidative damage by ROS [34]. Brain cells may particularly be at risk for oxidative stress. The brain derives its energy almost exclusively from oxidative metabolism through the mitochondrial respiratory chain, and is relatively deficient in protective mechanisms compared to other tissues such as liver and kidney. It contains reduced quantities of CAT, glutathione peroxidase (GPx), GSH and vitamin E than the liver or the kidney [31].

To define a cellular redox state, the ratio of interconvertible reduced/oxidized forms of molecules such as NAD^+/NADH, $NADP^+$/NADPH, and GSH/oxidized glutathione (GSSG) was employed. The ratio GSH/GSSG is the most important and commonly measured redox couple used to estimate the cellular redox state [35]. Under oxidative stress conditions GSH (reducing power) is decreased and GSSG is increased. Moreover, oxidative condition of a tissue might also be assessed by measuring the $A^{\bullet}/AH^{-}$ ratio that reflects the actual state of the oxidative defense system at the hydrophilic level, providing an early and simple diagnosis of stress [36-38]. To assess oxidative conditions in the hydrophilic cellular medium associated to As administration in brain, Wistar male rats were treated with a single ip dose of sodium arsenite ($NaAsO_2$) (5.8 mg As^{3+}/kg). After 24 h, the brain was isolated and As content was assayed according to Navoni *et al*. [39], and the detected content was 0.5 ± 0.1 μg/g of wet tissue. Data in Figure 2 show the EPR spectrum for brain $A^{\bullet}$ both in control and treated animals. AH^{-} content was determined by High-Performance Liquid Chromatography (HPLC-EQ) [40] and the $A^{\bullet}/AH^{-}$ ratio was calculated. The $A^{\bullet}/AH^{-}$ ratio was 2.3 ± 0.9 and 2.21 ± 0.06 AU for brain from control and treated rats, respectively.

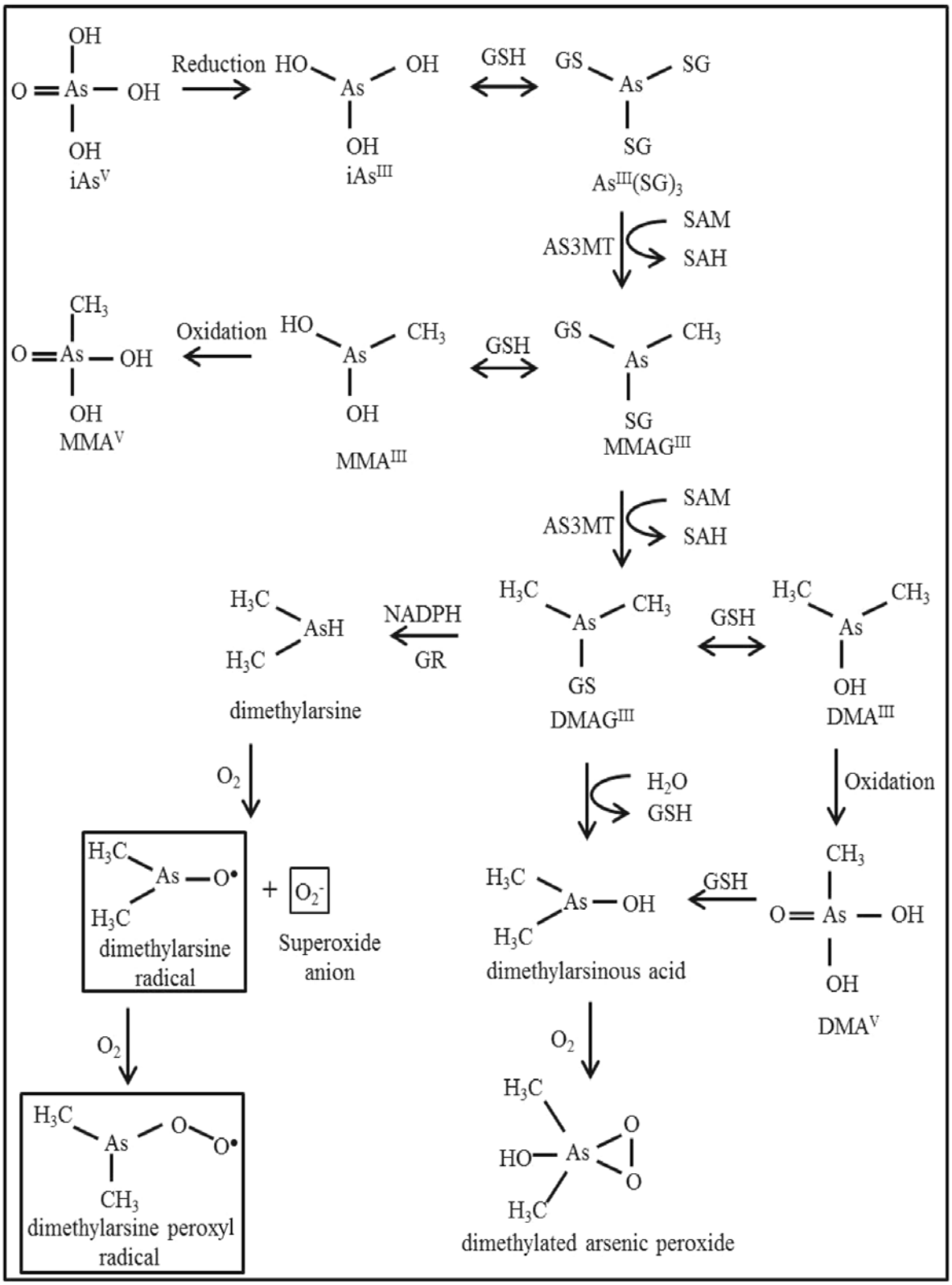

Figure 1. Metabolism of As showing the proposed formation of free radicals. Taken and modified from Hayakawa *et al*. [17] and Yamanaka *et al*. [26].

Table 1. Recent models applied to As-oxidative stress studies.

Model	Doses	Chemical administrated	Analysed Tissue	Main assays	Ref.
Isolated cell lines					
PLHC-1 Fish	0-100 µM	As_2O_3 at 10, 20 & 40 h		a, k	[67]
Human leukemia	0-5 µM	As_2O_3 at 3, 4, 6 & 24 h		a	[68]
SVEC4-10	0-7.5 µM	As_2O_3 at 24 h		a, l	[69]
BALB/c mice splenocytes	0-1 µM	As_2O_3 at 2 h		a, o	[70]
C2C1q2 mioblas	0-30 µM	As_2O_3 at 24 h		a, b, j	[71]
CD4-t from B10.D2 mice	10 µM	As_2O_3 at 5 h		a, c	[72]
Human hepatocellular carcinoma	0-20 µg/ml	As_2O_3 at 24 & 48 h		a, b, c, h, i, k	[9]
Caco-2	0-100 µM	iAs^{III}, iAs^{V}, MMA^{III}, MMA^{V}, DMA^{III}, DMA^{V} at 24, 48 & 72 h.		a, b, c, d, f, g, h, i, k	[8]
Human lung adenocarcinoma	0-100 µM	As_2O_3 & $NaAsO_2$ at 24 h		a, c, h	[73]
Murine neuroblastoma	0-10 µM	As_2O_3 at 24 h		a, b, k	[7]
Animal					
Goat	50 mg/kg food	$NaAsO_2$, 12 months	Blood	h, i, t	[74, 75]
Wistar Rat	0.4, 4 & 40 mg/l	$NaAsO_2$, 2 to 18 weeks	Blood & liver	b, j, p	[76]
Wistar Rat	5 mg/kg/day oral	$NaAsO_2$, 4 weeks	Blood & kidney	b, c, d, e, f, g, h, i, o	[77]
Wistar Rat	25 mg/l	$NaAsO_2$, 4, 8 & 12 weeks	Blood, liver & kidney	b, h, i, j, o	[78]
Wistar Rat	100 mg/l	$NaAsO_2$, 28 days	Brain, heart & liver	b, d, f, h, i	[43]
Wistar Rat	10 mg/kg oral	$NaAsO_2$, 10 days	Liver	b, d, e, f, h, i	[79]
Wistar Rat	100 mg/l	$NaAsO_2$, Gestational day 6 to 3 months	Brain	b, d, e, f, h, i, u	[51]
Wistar Rat	1 and 10 mg/l	$NaAsO_2$, 45 to 90 days	Blood & liver	b, c, f, h, i, j	[10]
Wistar Rat	3.8 mg/kg i.p.	As_2O_3, $NaAsO_2$, Na_2HAsO_2 and DMA^{V}, 24 h	Liver & kidney	b, b, d, f, i, q	[11]
Wistar Rat	7.5 mg/kg/day oral	$NaAsO_2$, 8 to 16 weeks	Blood & brain	a, b, h	[80]
Swiss mice	50 mg/l	$NaAsO_2$, 24 weeks	Blood, brain & liver	a, c, g, h	[81]
C3H/HeN mice	85 mg/l	$NaAsO_2$, 8 to 18 day of pregnancy	Liver	l	[82]
BALB/c and NUDE mice	1 mg/kg i.p.	As_2O_3, 7 days	CT26 tumor bearing	o, r	[70]
BALB/c mice	3 mg/kg/day i.p.	As_2O_3, 7 days	Liver	d, h, i, s	[83]
C57BL/6N mice	3, 6 & 10 mg/kg oral	$NaAsO_2$, 9 days	Blood, liver & spleen	b, c, f	[12]

The main assays reported are: a, oxidation of DCFH-DA; b, TBARS production; c, GSH content; d, GR activity; e, GST activity; f, GPx activity; g, GSSG content; h, SOD activity; i, CAT activity; j, Protein carbonyl content; k, Caspase 3 activity; l, Heme Oxygenase 1 activity; o, NO content; p, 8-OHdG content; q, Tiorredoxin reductase activity; r, Peroxynitrite ($ONOO^-$) content; s, Peroxidases activity; t, SOD's RNA and u, Mn-SOD activity.

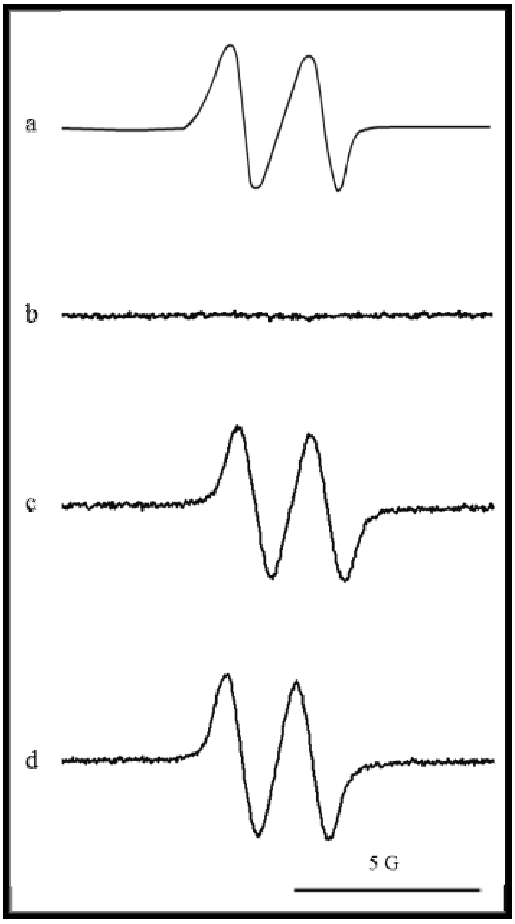

Figure 2. EPR spectrum for A$^{\bullet}$ in rat brain, (a) computer simulated spectrum employing the following spectral parameters: g = 2.005 and a_H = 1.8 G (g being the proportionality factor (g = $h\upsilon/\mu_B B_0$, where μ_B is the Bohr magneton and *Bo* is the magnetic field) with a value for a free electron of 2.00232, and a_H the protonic hyperfine spliting constant), (b) DMSO alone, (c) control rat brain, (d) As exposed rat brain at 24 h post-injection. DMSO stands for dimethylsulfoxide. A$^{\bullet}$ content in brain was determined according to Piloni *et al*. [65] using an EPR Bruker ECS 106 band X spectrometer.

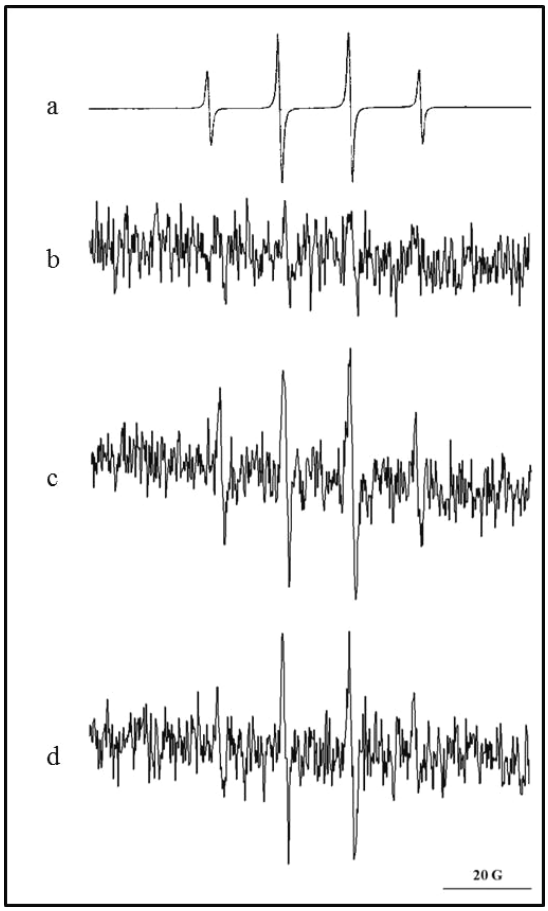

Figure 3. $^{\bullet}$OH generation by rat brain microsomes after acute As administration. EPR spectrum for $^{\bullet}$OH generation in rat brain, (a) computer simulated spectrum employing spectral parameters a_N = 15 G (a_N being the nitrogen hyperfine splitting constant) and a_H = 15 G; (b) basal system (in the absence of microsomes); (c) DMPO-OH spin adduct generated in rat brain microsomes from control animals; (d) DMPO-OH spin adduct generated in rat brain microsomes from treated animals. DMPO stands for 5,5-dimethyl-1-pyrroline n-oxide. The basic microsomal incubation system and the EPR spectrometer settings were as described by Malanga *et al*. [66].

Thus, no significant differences in the ratio between samples were observed.

Moreover, the rate of generation of $^{\bullet}$OH was determined by EPR in brain homogenates (Figure 3) and no significant differences were seen between control and treated animals at 24 h post-treatment. These results indicate that oxidative stress conditions have not been developed at the hydrophilic cellular environment. However, further experiments are required to assess a possible triggering of oxidative stress in the lipophilic medium in this animal model.

Polyunsaturated fatty acids are a major constituent of neural cell membranes and are substrates for free radicals and lipid peroxidation [41]. Low doses of arsenite chronically administrated over a period of time in drinking water increased lipid peroxidation in the brain of rats, accompanied by reduced antioxidant activity of the enzymes SOD

and GPx [42]. In this regard, Bharti *et al*. [43] showed that rats exposed to As (100 ppm sodium arsenite via drinking water for 28 days) had a marked elevation in lipid peroxidation in brain with a significant decrease in the activities of CAT, SOD, GPx, GR and GSH level.

Besides the important role of As as an oxidative stress generator, other actions may take place in the central nervous system (CNS) and contribute to the observed toxic effects of arsenicals [31]. For instance, in the basal ganglia arsenite exposure induces changes in neurotransmitter levels and alterations of functions reflected in behavioral tests [44]. This is a region with high Fe content, and it has been demonstrated that methylated As species cause the release of Fe from ferritin (Ft) *in vitro*, and that this "free" Fe can produce ROS [45]. There is another pathway through which arsenicals can release Fe, namely the activation of the enzyme heme-oxygenase (HO) [46]. Negishi *et al.* [47] reported that the adverse effects of developmental exposure to 20 mg/l diphenylarsenic acid (DPAA) in drinking water could be due to an increase in HO-1 in the cerebellar astrocytes.

Moreover, Ahmad *et al*. [45] suggested the direct reduction of Ft Fe by DMA^{III} as the predominant Fe release pathway, and that it is not mediated via $O_2^{\cdot-}$. These authors also suggested that DMA^{III} or DMA^{III} and ascorbic acid-mediated Fe-release from Ft may be even more significant *in vivo* because of three observations: (a) the large amount of Ft Fe release caused by a reductive chemical process, (b) no significant effective antagonism of SOD on DMA^{III} or on DMA^{III} and ascorbic acid-mediated Fe-release, and (c) strong stimulatory effect of CAT.

García Chavez *et al*. [28] analyzed the ability of arsenite to produce $^{\bullet}OH$ in the brain of awake, freely moving rats by assaying the free radical by an indirect method [48]. They reported significant differences over basal values only at 50 and 400 mM arsenite when mean responses to As at each sampling point were compared. Nevertheless, they did not observe a dose-response pattern and the time course of the response was very different among subjects. Mishra and Flora [49] showed that some aspects of As toxicity in the brain could be connected to the generation of $^{\bullet}OH$. To explain their results showing an increase in $^{\bullet}OH$ production, an interesting route to produce H_2O_2 was proposed by the oxidation of As(III) to As(V) which, under physiological conditions, resulted in the formation of H_2O_2, a source of the dangerous $^{\bullet}OH$ (eq 1).

$$H_3AsO_3 + H_2O + O_2 \longrightarrow H_3AsO_4 + H_2O_2 \quad \text{(eq 1)}$$

This reaction is spontaneous and exergonic with an estimated standard reaction free energy change ($\Delta_rG°$) for H_2O_2 formation of -40.82 kcal/mol (-170.87 J/mol) [50].

Recently, Kadeyala *et al*. [51] showed that As exposure significantly decreased the activity of SOD, CAT, GPx, and GR with increase in glutathione-S-transferase (GST) while lipid peroxidation, As levels, and mRNA expression of caspase 3 and 9 were significantly increased in different brain regions. Moreover, the authors indicated that cortex was the most susceptible region to As effect. Furthermore, Bashir *et al.* [52] proposed that apart from oxidative stress and necrosis, acute *in vivo* As exposure leads to induction of apoptosis as evidenced by the activation of caspases and formation of DNA ladder. In addition to ROS, As exposure can also initiate the generation of reactive nitrogen species (RNS) [50]. However, several conflicting reports concerning As-induced production of NO have been published [53]. Pachauri *et al*. [54] suggested that chronic As exposure caused a significant increase in ROS followed by NO and calcium influx, with a significant role for mitochondrial-driven apoptosis. As has also been found to initiate endothelial dysfunction by diminishing the integrity of vascular endothelium followed by inactivation of endothelium-Nitric Oxide Synthase (eNOS), thereby reducing the generation and bioavailability of NO and increasing oxidative stress [55].

5. As relevance in human health

As exposure affects millions of people worldwide. Epidemiological studies appear to provide a guide for As risk assessment in water, air and dust [55]. Although the toxic and carcinogenic effects on humans exposed to As have been well documented, the mechanisms by which As induces health damages are not well characterized. Data included in Table 2 briefly summarizes the main effects of As exposure on human health. The most recent epidemiological

Table 2. As and human diseases.

Diseases	Characteristics	Ref.
Hepatic and renal disorders	Positive correlation between chronic As exposure in humans and hepatotoxicity As causes acute renal failure and chronic renal insufficiency	[60, 84]
Cardiovascular disorders	Hypertension, QT prolongation, peripheral arterial disease, atherosclerosis, impaired microcirculation, coronary heart disease, stroke	[85]
Type 2 diabetes	Controversial data. Epidemiological reports of insulin resistance and reduced insulin secretion by Diaz-Villasenor *et al.* in As-exposed populations	[60, 86]
Carcinogenesis	Populations exposed to As are at risk of developing skin, bladder, liver and lung cancers	[87-89]
Gastrointestinal disturbances	Exposure to inorganic As produces clinical signs such as gastrointestinal irritation, nausea, vomiting, diarrhoea and abdominal pain in all cases of short-term high-dose and longer-term lower-dose exposure	[55, 90, 91]
Neurological defects	Depending on the dose, duration and route of As exposure its effects range from neurobehavioral disturbances to memory and cognitive impairments, visual or auditory sensory defects and peripheral neuropathies and encephalopathies	[60]

studies are mostly related to studies in blood and urine [56-58]. The effect of As on the GSH/GSSG ratio in humans was reported by Hall *et al*. [58], and damage to DNA by Ahmed *et al*. [56] and Pei *et al*. [59]. However, these studies were performed on different populations (from China, Taiwan, Bangladesh, etc.) exposed to As concentrations from 0.13 to 12.9 μM, limiting the possibility of global analyzis of the shown data.

As described above, induction of ROS and depletion of antioxidant defenses by As have been shown to be most important factors governing its toxic effect. A therapeutic strategy to increase the antioxidant capacity of cells may fortify the long-term effective treatment of As poisoning [60]. To accomplish this goal either the interaction of As with critical biomolecules should be reduced or the cells could be supplemented with antioxidant molecules. Many antioxidants (N-acetylcysteine (NAC), α-lipoic acid, α-tocopherol and vitamin C) have been tested as agents capable of reversing As-induced oxidative stress and related disorders. Moreover, quercetin, essential metals, natural/herbal antioxidants and chelation therapy have also been shown to be efficient protectors against As-dependent damage [60].

It is also widely recognized that As-induced formation of ROS and subsequent depletion of antioxidant cell defenses can result in disruption of the antioxidant/prooxidant equilibrium in mammalian tissues. Owing to its sulfhydryl group binding capacity, As can also inhibit the activities of many enzymes, especially those involved in the uptake of glucose in cells, fatty acid oxidation and production of GSH [55]. Biswas *et al.* [61] reported a protective role for the phytochemical curcumin against As-dependent DNA damage in a field trial undertaken in West Bengal.

The most effective treatment known for As poisoning is chelation therapy; however many of these compounds result in several undesirable effects [62]. Chelating agents are organic compounds capable of linking together As ions to form complex ring-like structures called chelates. Sodium 2,3-dimercaptopropane 1 sulfonate (DMPS), meso-2,3-dimercaptosuccinic acid (DMSA) and one of its analogues monoisoamyl-DMSA (MiADMSA) are effective chelators, and the dithiol groups in their moieties act as oxygen radical scavengers, which help in inhibiting lipid peroxidation [63]. DMSA and DMPS are effective in reversing As-induced toxicity since they enhance urinary As excretion and contribute to the restoration of hepatic GSH [64].

Although the toxic and carcinogenic effects on humans exposed to As have been well documented, many of the reported effects have been tested in animal models. Moreover, the mechanisms by which As induces cancer, cardiovascular disorders, and metabolic diseases are not well characterized [55].

6. CONCLUSION

Research work on As poisoning has revealed that free radical-mediated oxidative damage is a common denominator of As pathogenesis. In summary, it is now clear that introduction of As into a cell causes an imbalance between pro-oxidants and endogenous antioxidants that triggers various pathways to prime the cell towards apoptosis or immortality. Although the mechanism of As-induced toxicity still remains poorly understood, the mitochondria are presumed to be one of the major targets for the generation of reactive species, which trigger a cascade of events leading to cell death. The action of As-induced apoptosis is complex and H_2O_2 is believed to be involved in the process [60]. However the data shown here, employing EPR technology, clearly indicates that there is no significant effect of As in the cellular hydrophilic medium, at least under the experimental conditions tested here. Further studies should be developed to analyze the role of lipid peroxidation in this acute model of As toxicity.

This knowledge will be of great help in developing therapeutic strategies that, at this stage, seem to involve chelation therapy. Moreover, some other novel metabolic possibilities that would lead to As-induced oxidative stress and damage could include the participation of catalytic active metals such as Fe, which should be analyzed in the context of designing future treatments in humans. However, an important point to be taken into account is that animal models and humans do not metabolize As identically. Li *et al.* [92] identified large differences in the sequence of the enzyme AS3MT among humans, rat, and mouse. Moreover, Healy *et al.* [93], Wildfang *et al.* [94] and Del Razo [95] established differences in the biological activities of AS3MT in hamster, rabbit, rhesus monkey and rat. Drobná *et al.* [96] found variations in AS3MT expression, particularly in the amount of protein among species (rat, rhesus monkey, dog, mouse, rabbit and humans). These variations could explain both, the speed differences in methylation, and the changing ratio of methylated metabolites among species.

Man is the only mammal that excretes MMA in urine in larger amounts, which differs significantly from the other species [97]. Rat, rhesus monkey and dog (Beagle) were proven to have quick methylation of As compared with humans, rabbit and mouse (slow methylation), and in humans the intracellular retention time of As and its metabolites differs from rabbit and mouse [96]. The rat has a very efficient methylating capacity of As; nevertheless the DMA is not excreted, but stored in red blood cells [98]. These features point to the need for proceeding very carefully when using these models to develop protocols to be applied in humans.

ACKNOWLEDGMENTS

These studies were supported by grants from the University of Buenos Aires, ANPCyT and CONICET. S.P. is a career investigator from CONICET and J.G.B. is a fellow from ANPCyT. The authors are grateful to Dr. Elizabeth Robello for her help with the EPR studies reported here.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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