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Arsenic-induced neurotoxicity: A mechanistic appraisal

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Abstract

Arsenic is a metalloid found in groundwater as a byproduct of soil/rock erosion and industrial and agricultural processes. This xenobiotic elicits its toxicity through different mechanisms, it has been identified as a toxicant that affects virtually every organ or tissue in the organism. In the central nervous system exposure to arsenic can induce cognitive dysfunction, it has been linked to several neurological disorders, including neurodevelopmental alterations, and is considered a risk factor for neurodegenerative disorders. However, the exact mechanisms involved are still unclear. In this review, we aim to appraise the neurotoxic effects of arsenic and the molecular mechanisms involved. First, we discuss the epidemiological studies reporting on the effects of arsenic in intellectual and cognitive function during development as well as studies showing the correlation between arsenic exposure and altered cognition and mental health in adults. The neurotoxic effects of arsenic and the potential mechanisms associated with neurodegeneration are also reviewed including data from experimental models supporting epidemiological evidence of arsenic as a neurotoxicant. Next, we focused on recent literature regarding arsenic metabolism and the molecular mechanisms that begin to explain how arsenic damages the central nervous system; including, oxidative stress, energy failure and mitochondrial dysfunction, epigenetics, alterations in neurotransmitter homeostasis and synaptic transmission, cell death pathways and inflammation. Outlining the specific mechanisms by which arsenic alters the cell function is key to understand the neurotoxic effects that convey cognitive dysfunction, neurodevelopmental alterations and neurodegenerative disorders.

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Conflict of Interest statement

The authors declare that they have no conflict of interest.

Keywords

iAs; redox homeostasis; oxidative stress; metalloid; neurotoxicity

Introduction

Arsenic (*As*) is an element cataloged as a metalloid, because it has both metal and nonmetal properties. This metalloid is also classified as a xenobiotic because it does not have physiological functions but exerts toxic effects on humans. The principal route of exposure to this metalloid is through the food and contaminated water. In the World, there are a number of regions where the levesl of *As* in drinking-water exceeds the recommended exposure limits. At least 140 million people in 50 countries have been drinking water containing *As* at levels above 10 μg/L (world Health Organization [WHO] provisional guideline). *As* is naturally present at high levels in the groundwater of a number of countries, including Argentina, Bangladesh, Chile, China, India, Mexico, and the United States of America. *As* has been identified as a toxicant that affects virtually every organ or tissue in the organism, its deleterious effects include, skin lesions, different types of cancer, as well as cardiovascular, respiratory and gastrointestinal effects. In the nervous system this metalloid can induce peripheral neuropathies, encephalopathy, neurobehavioral alterations, and has also been associated to neurodegeneration. Although there are toxicological and epidemiological studies that show the neurotoxic effects of *As*, this topic is still evolving [1-3].

Environmental and anthropogenic sources of Arsenic

As is present in soil, air and water, it is widely distributed in the Earth's crust being the 12th most abundant element in the human body. *As* compounds can be categorized as inorganic (*iAs*) and organic. *iAs* occurs in two oxidation states, arsenite +3 (*iAsIII*) and arsenate +5 (*iAsV*). Nowadays *iAs* is used as a wood preservative, in agricultural chemicals, as an alloying element, in the electronics industry, and it has been used to treat leukemia. At least 140 million people in 50 countries have been exposed to *iAs* at levels above the WHO recommended exposure limits (10 ppb). In contaminated areas *iAs* concentration can exceed 1,000 ppb in groundwater [4, 5].

Toxic effects of Arsenic in the Nervous System

Gestational and developmental effects

A clear understanding of the gestational and developmental neurotoxicity of *iAs* is still missing. However, toxicological and epidemiological reports have demonstrated that exposure to *iAs* during development affects intellectual and cognitive function (Table 1). Furthermore, a reduction in full-scale intelligence quotient (IQ) and memory have been linked to *iAs* exposures even below current safety recommendations [6, 7]. Children exposed to *iAs* concentrations ranging from 5 to 50 ppb in water, from Mexico [8, 9], the US [10] and Bangladesh [11] showed neurobehavioral alterations in verbal abilities, cognitive function, IQ, motor skills and long term memory. Gestational exposure to sodium arsenite

($NaAsO_2$) increases its accumulation in the mice offspring's brain and impairs learning and memory processes [12, 13]. Additionally, prenatal exposure of mice to $NaAsO_2$ produces behavioral impairments as well as abnormal formation of the prelimbic cortex in the adult offspring [14].

Adults

Epidemiological reports on the effect of *iAs* exposure on adults are limited (Table 1). A series of studies have shown a significant correlation between *iAs* exposure and altered adult cognition and mental health [15, 16]. High levels of *iAs* in drinking water and/or urine in adults have been associated with peripheral nerve disturbances, decreased peripheral nerve conduction velocity, peripheral neuropathy, and altered sensory function [2, 17-19]. After ingestion of a single dose of *iAs*, four patients developed a peripheral neuropathy showing reduction of motor conduction velocity and marked abnormalities of sensory nerve action potentials [20]. Disappearance of neurofilament and fibroblast proteins in the sciatic nerves occurred in rats exposed to *iAs* [21, 22]. Moreover, *iAs* exposure in rats increases oxidative damage, demyelination, and morphological alterations in axons of peripheral nerves, suggesting that these changes can lead to decreased transmission of information between peripheral sensory organs to the central nervous system (CNS) [23].

Neurodegeneration

Several association studies have failed to demonstrate that exposure to *iAs* alone causes neurodegeneration. However, the neurotoxic effects of *iAs* can correlate or synergize with the molecular mechanisms associated with neurodegeneration (oxidative stress, mitochondrial dysfunction and inflammation). In a case-control study, an increased risk to develop AD was associated with urinary levels of *iAs* (high), dimethylarsinic acid (*DMAV*, low) and selenium (Se, low) [24], *iAs* can induce vascular injury and dementia *in vivo* [25]. In rats, chronic *iAs* exposure induces behavioral deficits accompanied by higher levels of advanced glycation end products (RAGE), increased amyloid-β production and β-secretase (BACE-1) activity [26]. *iAs* exacerbates amyloid-β and phosphorylated Tau immunostaining in transgenic Alzheimer's disease mouse models and this correlated with bioenergetic dysfunction and changes in redox metabolism [27]. The pro-amyloidogenic effects of *iAs* seem to be increased when combined with other heavy metals, and these effects seem to correlate with oxidative damage and neuroinflammation [28]. Accordingly, *iAs* increases the levels of pro-inflammatory cytokines in astrocytes that correlate with higher levels of amyloid precursor protein (APP) and BACE-1 [29], *iAs* can synergize with dopamine to trigger neurotoxicity [30]. *iAs* also induces the accumulation of α-synuclein (the pathological biomarker of Parkinson's disease) accumulation and oligomerization with an overall increase in biomarkers markers of proteotoxic stress, but did not trigger neurodegeneration *in vivo* [31]. These findings suggest that *iAs* exposure may increase the susceptibility of developing neurodegeneration.

Arsenic metabolism

Transport

iAs can be transported across the blood brain barrier (BBB) and enter the brain. *iAsIII* uses aqua(glycerol)porins (AQP), organic anion transporters and glucose transporters (GLUT) to enter the cell. While *iAsV* is transported through phosphate transporters and then, inside the cell, is reduced to *iAsIII* (Figure 1a) [32-34]. Once inside the brain *iAs* is methylated and accumulated, with the highest accumulation in the pituitary gland [35]. *iAs* metabolites are exported through the multidrug resistance proteins (MRP1, MRP2 or MRP4) (Figure 1d) [36-38]. Our results (*unpublished data*) together with other studies have shown that astrocytes can be resistant to *iAsIII* through the MRP proteins [39]. Interestingly, knockout mouse for the P-glycoprotein showed a higher accumulation of *iAs* in the brain [40].

Redox metabolism and methylation

Once inside the cell *iAsIII* can be methylated by different mechanisms. The first mechanism is the oxidative methylation that is mediated by arsenite methyltransferase (AS3MT) that uses S-adenosylmethionine (SAM) as a co-substrate. *iAsIII* is methylated to monomethylarsonic acid or arsonate (*MMAV*) that is reduced to monomethylarsonous acid (*MMAIII*) and then methylated again to dimethylarsinic acid (*DMAV*) [2, 41]. *DMAV* reduction generates dimethylarsinous acid (*DMAIII*) (Figure 1b). The pentavalent arsenicals (*iAsV, MMAV* and *DMAV*) can be reduced, through a process mediated by the thioredoxin/thioredoxin reductase (Trx/TR) system, although the antioxidant glutathione (GSH) seems to also increase their methylation by an unidentified mechanism [42].

Another mechanism for *iAs* methylation is via its conjugation with GSH where the GSH complexes generated are known as As triglutathione [$As(GS)_3$]. This conjugation can occur non-enzymatically or enzymatically via the glutathione-S transferases GSTO1, GSTM1 or GSTP1 [36, 41]. The $As(GS)_3$ complexes are methylated by the AS3MT to form monomethylarsinic diglutathione [$MMA(GS)_2$] and then again to generate dimethylarsinic GSH [*DMA(GS)*]. When GSH levels are low, the conjugates can be hydrolyzed and then oxidized to generate *MMAV* and *DMAV* (Figure 1c) [41].

iAsV has been shown to replace phosphate in several metabolic pathways in a process called arsenylation where the end product is the reduction of *iAsV* to *iAsIII* as the arsenylated byproduct is more readily reduced than *iAsV*, increasing *iAs* toxicity. *iAsV* uncouples oxidative phosphorylation and ATP formation in the mitochondria by binding to ADP via the ATP-synthase. Likewise, arsenylation during glycolysis can impair carbon flux and ATP production. Furthermore, *iAsV* reacts with glucose generating glucose 6-arsenate an analog of glucose 6-phosphate that can act as an inhibitor of the hexokinase. *iAsV* is arsenylated by a number of different enzymes [43-45]; therefore, alterations in central carbon metabolism, energy failure and mitochondrial dysfunction can also be consequences of *iAsV* toxicity (Figure 2a).

It is important to mention that while the primary organ involved in *iAs* methylation is the liver, significant amounts of *iAs* and methylated *As* forms can be found in mice exposed to *iAs. iAs* crosses the blood-brain barrier (BBB) and is methylated in different brain regions

that express AS3MT [35, 46]. Potentially, methylated *iAs* species in circulation can also cross the BBB but the mechanisms involved have not been studied. During development, *iAsV* or *iAsIII* might be accumulated in the brain more readily when the blood brain barrier is still not fully developed. In addition, iAs has been reported alter BBB gap junction formation as well [47, 48].

Thiol-binding properties

Recently, a third mechanism for *iAsIII* metabolism was proposed suggesting that first *iAsIII* binds to protein thiols and then it is methylated. This idea is supported by the findings that *iAsIII* has more affinity for protein thiols than for GSH [49]. *iAsIII* binds to thiol containing molecules and protein-cysteine thiols that can lead to enzyme inactivation. Moreover, the *iAsIII*-GSH complexes can bind to protein thiols, so the export of *iAs*- GSH adducts from the cell is an important process of detoxification [50]. Dithiol molecules together with proteins that have adjacent cysteines have been reported to bind *iAsIII* (Figure 2b) [51-55].

Molecular mechanisms of toxicity

Mitochondrial Dysfunction and Oxidative Stress

In the cell, a major source of reactive oxygen species (ROS) is the electron leak from the mitochondrial electron transport chain. Under physiological conditions, the steady-state levels of mitochondrial ROS are maintained by antioxidant systems, but in damaged or aged an increased ROS formation occurs [56-58]. Other sources of ROS / reactive nitrogen species (RNS) are the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidases (NOX) and the NO synthases (NOS). [59, 60] and to a lesser extent, the activity of enzymes such as xanthine oxidases, cyclooxygenases, cytochrome p450 enzymes, lipoxygenases, myeloperoxidases and the protein folding machinery in the endoplasmic reticulum (ER) [56, 57, 61]. Physiological levels of these reactive species participate in signaling pathways, however, an imbalance between an increase in the levels of ROS/RNS and their metabolism or detoxification leads to a state known as oxidative stress. When oxidative stress is generated it can lead to oxidative modifications of biomolecules associated with the loss of function of proteins, damage to organelles and even cell death [57, 58, 61, 62]. To prevent the generation of oxidative stress, cells have antioxidant compounds that can be classified as enzymatic and non-enzymatic [56-58, 61].

Due to the high levels O_2 consumption, the low content of antioxidants defenses, together with elevated levels of lipids and fatty acids, the brain is remarkably sensitive to oxidative damage. [63, 64]. In brain regions of animal models and in glial cell and neuron cultures exposed to iAs an increase in oxidative stress has been shown [65-69]. Mitochondria are a primary source of ROS formation induced by As [70, 71]. Accordingly, it has been shown that chronic iAs exposure increases mitochondrial oxidative stress in the rat brain by damaging the mitochondrial complexes I, II, and IV followed by increased ROS formation, protein carbonylation and lipid peroxidation [72]. Furthermore, the levels of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and the mitochondrial transcription factor A (TFAM) are reduced by iAs exposure leading to a decrease in mitochondrial biogenesis [73]. Dimethylarsine (DMAH) and peroxyl radicals can also be

generated by *iAs* and in turn mediate lipid peroxidation and the accumulation of oxidized byproducts. Moreover, the metabolites *MMAIII* and *DMAIII* appear to be more potent toxicants because their ability to produce radicals (Figure 2c) [74].

iAs also activates the antioxidant transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2), through a non-canonical pathway where inhibition of autophagy leads to the accumulation of the ubiquitin-binding protein/adaptor autophagic receptor sequestosome-1, also known as p62 (p62/SQSTM1) that sequesters the Nrf2 suppressor kelch-like ECH-associated protein 1 (Keap1) (Figure 2d) [75], *iAsIII* toxicity can be counteracted by the transcriptional regulation of metallothionein I via the metal-activated transcription factor 1 (MTF1) (Figure 2d) [76].

Epigenetics

Both *iAs* biotransformation by AS3MT and the salvaging of sulfur to maintain adequate redox buffering capacity via the transsulfuration pathway consume SAM (Figure 3a). SAM is also required for methylation-dependent epigenetic changes such as DNA-methylation and histone modification (Figure 3b). Thus, *iAs* exposure is known to affect the epigenetic control of gene expression. Several studies have investigated the epigenetic effects or *iAs* exposure in the brain during development. DNA methylation typically acts to repress gene transcription. In an epidemiological study, >2000 genes were identified with *iAs*-associated differences in DNA methylation in newborn cord blood. These changes were located primarily within CpG islands positioned within the first exon (regions of DNA susceptible for cytosine methylation to 5-methylcytosine, where a cytosine is followed by a guanine nucleotide), the 5' untranslated region and 200 bp upstream of the transcription start site suggesting a significant association with changes in gene expression. *iAs*–induced changes in gene methylation were related to changes in gestational age and head circumference [77]. Genes involved in neuroplasticity have altered methylation patterns during developmental exposure of rats to *iAs* [78]. Interestingly, brain tissue from weaned rats treated with *iAs* showed suppressed levels of DNA methyltransferases (DNMTs) and ten-eleven translocations (TETs), enzymes involved in DNA methylation and demethylation processes, respectively. These changes correlated with impaired memory and learning abilities. Importantly, SAM levels where unchanged suggesting that oxidative stress was primarily involved in the perturbations to DNA methylation processes [79].

Post-translational modification of histones modifies gene expression by altering chromatin structure and as a consequence DNA accessibility to the transcription machinery. Methylation and demethylation of histones turns genes "off" and "on," respectively (Figure 3b). On the other hand, acetylation of histones is known to increase the expression of genes. *iAs* alters mitochondrial function and metabolism, more specifically, it impairs pyruvate dehydrogenase (PDH) activity [80, 81], which catalyzes the oxidation of pyruvate to acetyl-CoA (Figure 3c). Acetyl-CoA is a required substrate for histone acetylation and it is also expected that *iAs* exposure will affect histone acetylation. In mice prenatally exposed to *iAs*, hypo-acetylation at histone 3K9 (H3K9) which correlated with impaired spatial and episodic memory [82]. In contrast, other studies have reported a sex-specific regulation of H3K9 acetylation and methylation in response to developmental *iAs* exposure [83].

Alterations in neurotransmitter homeostasis and synaptic transmission

iAs neurotoxicity has also been linked to changes in neurotransmitter metabolism leading to changes in the synaptic transmission [12, 13]. Realgar, an *As* sulfide mineral, and source of highly toxic *iAs*, increases extracelluar glutamate levels and induces excitotoxicity and changes in GLT-1 and glutamate / N-methyl-D-aspartate (NMDA) receptor levels in rat hippocampus [84]. Subtoxic *iAs* exposure downregulates the GluA1 subunit of the glutamate / α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor [85]. Gestational exposure of mice to iAs induces alters synaptic plasticity (longterm potentiation [LTP]), learning and memory that were associated with an increase in extracellular glutamate levels and downregulation of both AMPA and NMDA receptor subunits [12, 13], *iAs* also decreases the expression levels of α7 nicotinic receptors in rats [86], and alters development of the cholinergic and dopaminergic systems [87].

Arsenic (In microglia, *iAs* activates the cystine/glutamate exchanger system (xCT) to increase extracellular glutamate levels [88], whereas in astrocytes *iAs* decreases the expression and activity levels of glutamine synthetase (GS) and glutamate transporters (GLAST/EAAT1 and GLT-1/EAAT2) [66, 89]. Notably, an increase in GSH levels and Nrf2 activity but no oxidative stress, was also shown [66].

Cell death pathways

We will now discuss the activation of neural death pathways by *iAs. iAs* and its methylated metabolites induces caspase-dependent apoptosis in neurons and neuroblastoma cells through the mitogen-activated protein kinase (MAPK) signaling, that can involve the extracellular-signal-regulated kinase 2 (ERK2), p38 or c-Jun N-terminal kinases (JNK) [90, 91], Apoptosis induced by *iAs* can also be triggered by Ca^{2+} (Figure 4a) [92]. In vivo, *iAs* has been reported to trigger apoptosis in cerebral cortex [93]. Interestingly, *iAs*-induced apoptosis in hippocampal neurons seems to also me mediated by the antagonism of neurotrophic signaling [94].

Autophagy is a homeostatic process in which double–membraned autophagosomes engulf cellular components to be subsequently degraded upon fusion with lysosomes. Autophagosome cargo degradation preserves cellular homeostasis and viability via the turnover of damaged organelles and biomolecules whose prevalence or accumulation within cells can lead to deleterious effects. In most cases, induction of autophagy in response to stress acts as a pro-survival mechanism, but several examples have been reported where autophagy mediates cell death [95]. *iAs* treatment during development induces autophagy in the mouse brain via inhibition of phosphoinositide 3-kinase (PI3K) / Akt / mechanistic target of rapamycin (mTOR) signaling [48]. Inhibition of autophagy decreases the toxic effects of i*As* in glioblastoma cell lines [96]. We have observed that *iAs* induced apoptosis in cortical astrocytes is regulated by the AMP-dependent protein kinase (AMPK) / mTOR signaling pathways and autophagy activation (*unpublished data*) (Figure 4b). In a recent study, intra-hippocampal injection of low concentrations of $NaAsO_2$ (5 and 10 nM) enhanced autophagy and diminished apoptosis, while higher concentrations (100 nM) of $NaAsO_2$ showed the opposite effect. At low concentration *iAs* facilitated the acquisition of spatial learning and

the authors suggested that this phenomenon could be attributed to the induction of neuronal autophagy [97].

Inflammation

iAs induces strong inflammatory responses in the brain. In rat hippocampus, cultured microglia and astrocytes, *iAs* treatment increases the expression levels of the pro-inflammatory cytokines interleukin 1 beta (IL-1β), IL-6, interferon gamma (IFN γ) and TNFα [29, 98, 99]. Importantly, released cytokines can further mediate neuronal toxicity [99]. Chronic exposure with *iAs* also induces nitrosative stress by activating the inducible NOS (iNOS) in the brain. [100].

Conclusions and Perspectives

Understanding the neurotoxic effects of *iAs* is an important issue since *iAs* exposure is a public health concern around the World. The epidemiological reports undoubtedly show that *iAs* affects intellectual and cognitive function during development and in adults. Moreover, while *iAs* cannot be considered by itself a trigger of neurodegeneration there is evidence that suggests that it can increase the susceptibility to develop neurodegenerative disorders. While there are toxicological and epidemiological studies that show the neurotoxic effects of *iAs* the mechanisms involved are still unclear. In this review, we have summarized the current state of knowledge in regards to *iAs* metabolism and transport, and our current understanding of the molecular mechanisms involved in its neurotoxic effects. As summarized elsewhere [3], it is clear that more research is needed to clearly identify the mechanisms involved in the alterations in neurotransmission and cognitive/behavioral functions associated with developmental *iAs* exposure and the integrated role of neural cell populations (neurons and glia). In particular, a detailed exploration of the changes in epigenetic signatures and metabolism is still missing (bioenergetics, redox and neurotransmitter), which has the enormous potential to be used as biomarkers of disease mechanisms. Furthermore, the effect of aggregate exposures to the neurotoxicity of *iAs* has been poorly studied.

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List of abbreviations:

Aβ	amyloid beta peptide
AD	Alzheimer's disease
Akt	Protein Kinase B
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

AMPK	AMP-dependent protein kinase
APP	amyloid precursor protein
AQP	aqua(glycerol)porins
As	arsenic
$As(GS)_3$	arsenic triglutathione
As_2O_3	arsenic trioxide
AS3MT	arsenite methyltransferase
AsIII	arsenite
AsV	arsenate
BACE-1	β-secretase
BBB	blood brain barrier
BHMT	betaine hydroxy methyltransferase
Ca	calcium
Cd	Cadmium
CBS	cystathionine beta synthase
CNS	Central Nervous System
CoA	coenzyme A
COX-2	cyclooxygenase-2
CTH	cystathionine-γ-lyase or γ-cystathionase
Cys	cysteine
DMAIII(GS)	dimethylarsinic GSH
DMAIII	dimethylarsinous acid
DMAV	dimethylarsinic acid
DMAH	dimethylarsine
DMA•	dimethylarsine radical
DMAOO•	dimethylarsine peroxyl radical
DMAOOH	dimethylated arsenic peroxide
EAAT	excitatory amino acid transporter
ER	endoplasmic reticulum

ERK	extracellular-signal-regulated kinase
γ-GCL	gamma-glutamylcysteine ligase (or synthase; γ-GCS)
G3P	glyceraldehyde 3-phosphate
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GLUT	glucose transporters
GS	glutamine synthetase
GSH	glutathione
GSTs	glutathione-S transferases
HAT	histone acetyltransferases
iAs	inorganic As
iAsV-3-P-glycerate	1-arsenato-3-phospho-D-glycerate
IFNγ	Interferon gamma
IQ	intelligence quotient
IL-1β	interleukin 1 beta
iNOS	inducible NOS
JNK	c-Jun N-terminal kinase
Keap1	kelch-like ECH-associated protein 1
Pb	Lead
LTP	long-term potentiation
MAPK	mitogen-activated protein kinase
MAT	methionine adenosyltransferase
5,10-MeTHF	5,10-methylene tetrahydrofolate
MeTs	methyltransferases
2-MG	2-methyl glycine
$MMA(GS)_2$	monomethylarsinic diglutathione
MMAIII	momomethylarsonous acid
MMAV	monomethylarsonic acid
MRPs	multidrug resistance proteins
MS	methionine synthase

MT	metallothionenin
MTF1	metal-responsive transcription factor-1
5-MTHF	5-methyl tetrahydrofolate
MTHFR	methyl tetrahydrofolate reductase
mTOR	mechanistic (or mammalian) target of rapamycin
$NaAsO_2$	sodium arsenite
NADPH	nicotinamide adenine dinucleotide phosphate
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	glutamate/N-methyl-D-aspartate
NMDAR	glutamate/N-methyl-D-aspartate receptor
NO	nitric oxide
NOS	nitric oxide synthase
NOX	NADPH oxidases
NQO1	NADPH dehydrogenase quinone 1
Nrf2	nuclear factor (erythroid-derived 2)-like 2
p62	autophagic receptor sequestosome-1, p62/SQSTM1
PD	Parkinson's disease
PDH	pyruvate dehydrogenase
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PI3K	class III phosphatidylinositol 3-kinase
PNS	peripheral nervous system
Pi	phosphate
PPi	diphosphate
PTGEs	prostaglandin E synthase
RAGE	advanced glycation end products
RNS	reactive nitrogen species
ROS	reactive oxygen species
SAH	s-adenosylhomocysteine

SAHH	SAH hydrolase
SAM	S-adenosylmethionine
SOD	superoxide dismutases
TCA	tricarboxylic acid
TFAM	mitochondrial transcription factor A
TNF-α	tumor necrosis factor alpha
TR	Trx reductase
Trx	thioredoxin
xCT	cystine/glutamate exchanger system

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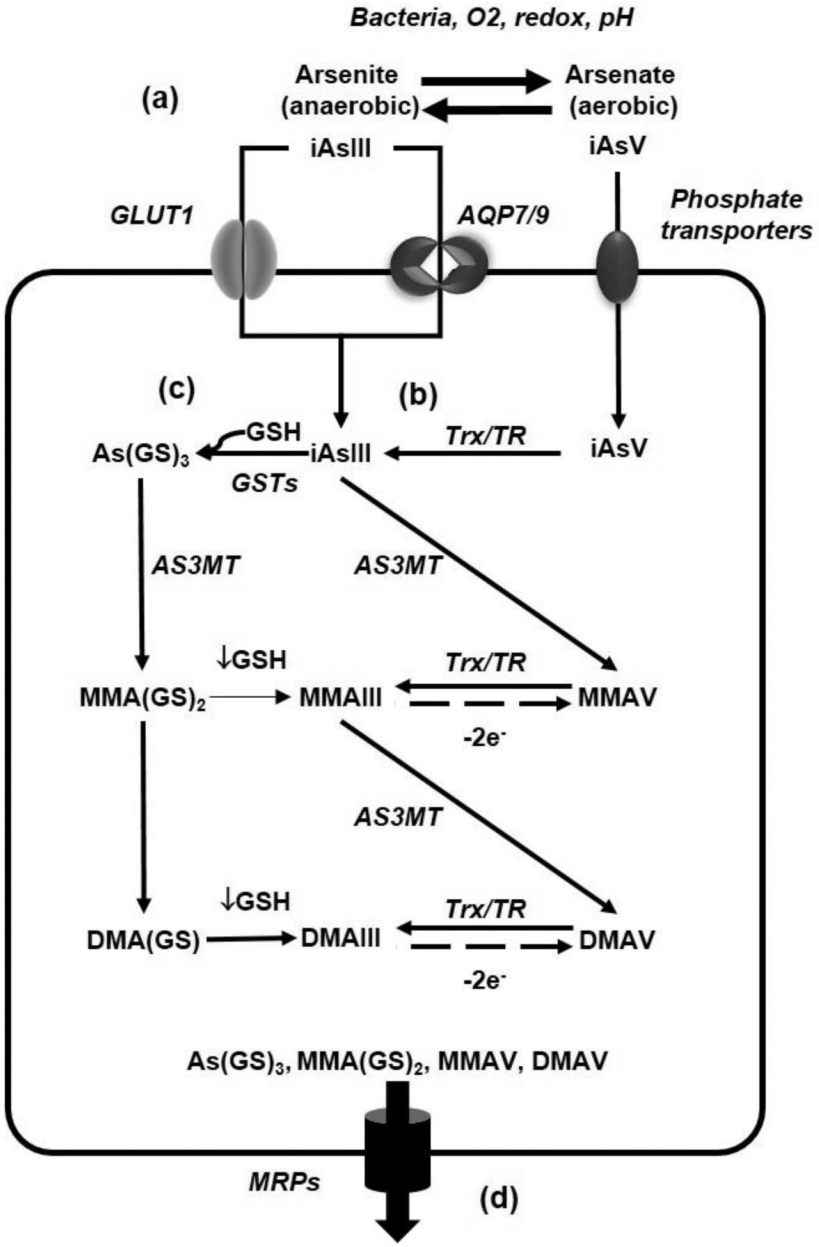

Figure 1. *iAs* metabolism.

(a) Arsenite (*iAsIII*) enters the cells through aqua(glycerol)porins (AQP) and the glucose transporters (GLUT), while arsenate (*iAsV*) does it through phosphate transporters. *iAs* is methylated through different mechanisms: **(b)** Oxidative methylation refers to the reduction of *iAsV* to *iAsIII* by the thioredoxin (Trx) / trx reductase (TR) system, and its subsequent methylation by the arsenite methyltransferase (AS3MT). Monomethyl arsonous acid (MMAIII), monomethylarsonic acid (*MMAV*); dimethylarsinous acid (*DMAIII*) and dimethylarsinic acid or cacodylic acid (*DMAV*) are the metabolites formed, **(c)** The glutathione (GSH) conjugation mechanism is based on the formation of GSH complexes with *AsIII* resulting in the formation of arsenic triglutathione [*As(GS)3*] by glutathione S-transferases (GSTs). The $As(GS)_3$ is subsequently methylated by AS3MT to form

monomethylarsinic GSH [*MMA(GS)$_2$*] and dimethylarsinic GSH [*DMA(GS)*]. **(d)** *iAs* metabolites are exported through the multidrug resistance proteins (MRP1, MRP2 or MRP4)

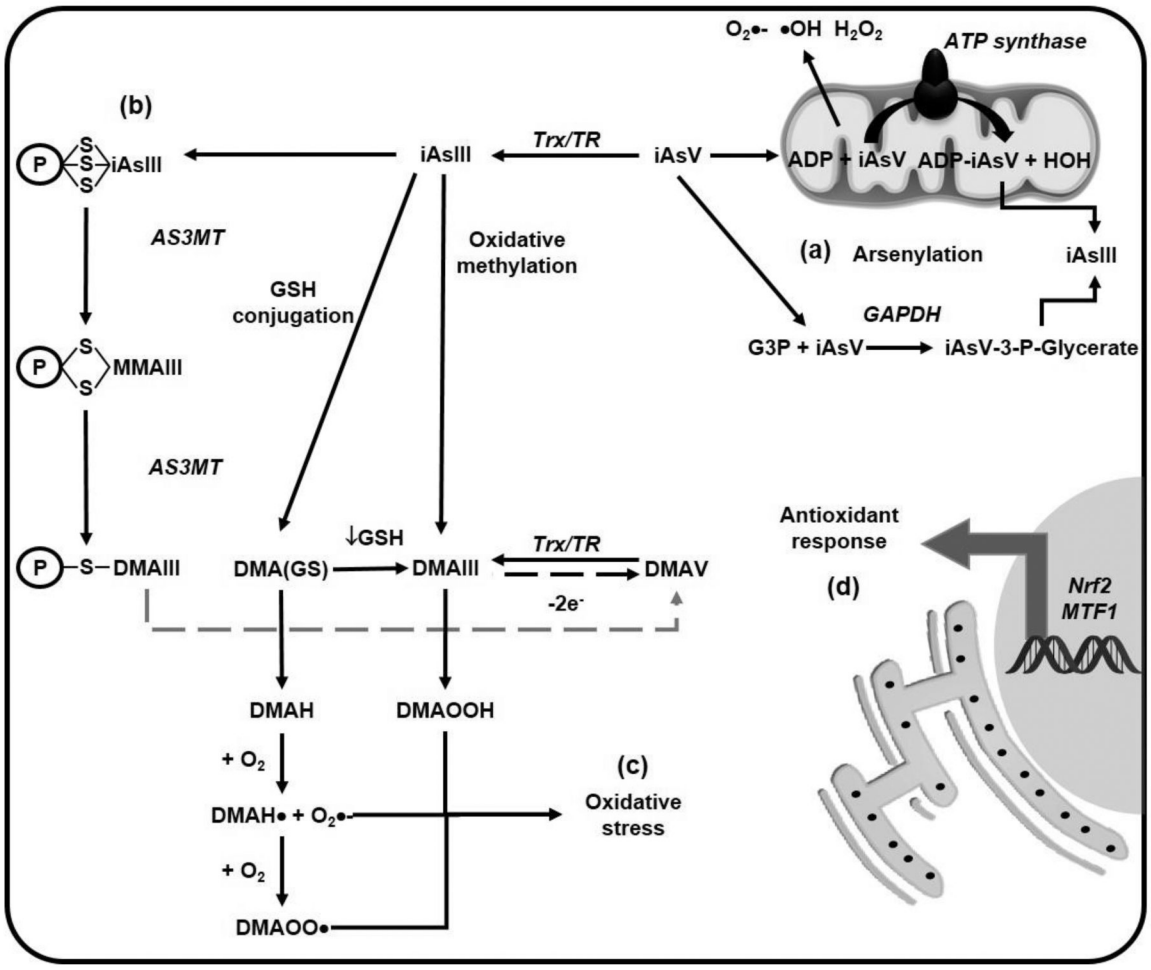

Figure 2. Alterations in mitochondrial function and redox balance induced by *iAs*.

(a) Arsenate (*AsV*) can replace phosphate in several metabolic pathways (arsenylation) where the end product is the reduction of *AsV* to arsenite (*AsIII*). *AsV* uncouples oxidative phosphorylation and ATP formation in the mitochondria by binding to ADP via the ATP-synthase. *AsV* is also arsenylated by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to generate 1-arsenato-3-phospho-D-glycerate (iAsV-3-P-glycerate) from glyceraldehyde 3-phosphate (G3P). **(b)** *iAsIII* binds to protein thiols (coenzyme A, lipoic acid and zinc finger domain containing proteins such as metallothionenin [MT]) and is methylated while still conjugated to these proteins, **(c)** Dimethylarsinic GSH [*DMA(GS)*] can form dimethylarsine (*DMAH*) and react with molecular oxygen (O_2) to form *DMAH* radical (*DMAH•*) and the DMAH peroxyl radical (*DMAOO•*). Dimethylarsinous acid (*DMAIII*) reacts with O_2 and forms dimethylated arsenic peroxide (*DMAOOH*). These peroxyl radicals lead to lipid peroxidation and protein carbonylation (oxidative stress), **(d)** *AsIII* induces an antioxidant response via nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and metal-responsive transcription factor-1 (MTF1).

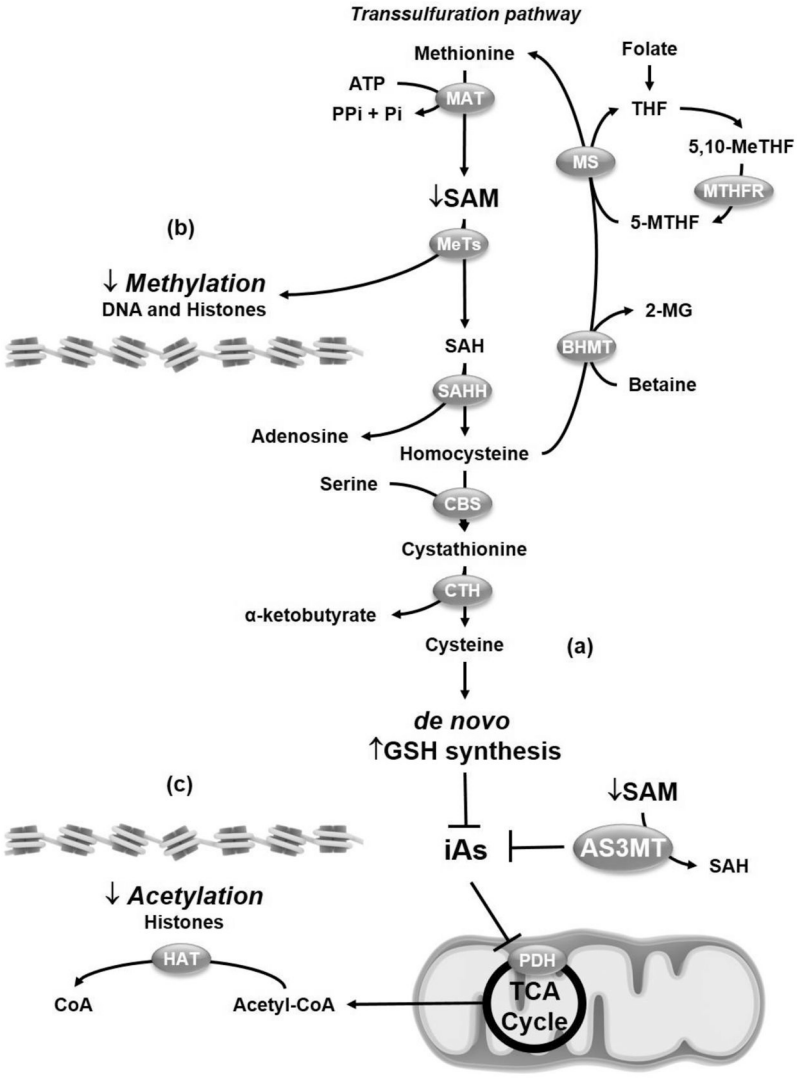

Figure 3. Epigenetic changes induced by *iAs*.

(a) *iAs* detoxification by arsenite methyltransferase (AS3MT) and glutathione (GSH) requires adenosylmethionine (SAM) consumption. In the transsulfuration pathway methionine is converted to SAM in an ATP-dependent reaction catalyzed by methionine adenosyltransferase (MAT). Subsequently, S-adenosylhomocysteine (SAH) is generated via the activity of a number of methyltransferases (MeTs). SAH is hydrolyzed by SAH hydrolase (SAHH) to adenosine and homocysteine. Homocysteine is conjugated with serine to provide cystathionine by the action of cystathionine beta synthase (CBS). Finally, cystathionine-γ-lyase (CTH, or γ-cystathionase) catalyzes the conversion of cystathionine to cysteine, which is used for *de novo* GSH synthesis, **(b)** If homocysteine is converted to cysteine and cannot be recycled re-methylated to methionine via the activities of betaine hydroxy methyltransferase (BHMT) or methionine synthase (MS) the SAM pool is depleted. SAM depletion will alter both DNA and histone methylation and the epigenetic signature in the brain that determines the expression of genes that might affect the neurodevelopmental processes. **(c)** Similarly, inhibition of pyruvate dehydrogenase (PDH) by *iAs* will impair

Acetyl-CoA synthesis and as a consequence the acetylation of histones by histone acetyltransferases (HAT) and the epigenetic regulation of genes. CoA, coenzyme A; 5,10-MeTHF, 5,10-methylene tetrahydrofolate; 2-MG, 2-methyl glycine; 5-MTHF, 5-methyl tetrahydrofolate; MTHFR, methyl tetrahydrofolate reductase; Pi, phosphate; PPi, diphosphate; TCA, tricarboxylic acid.

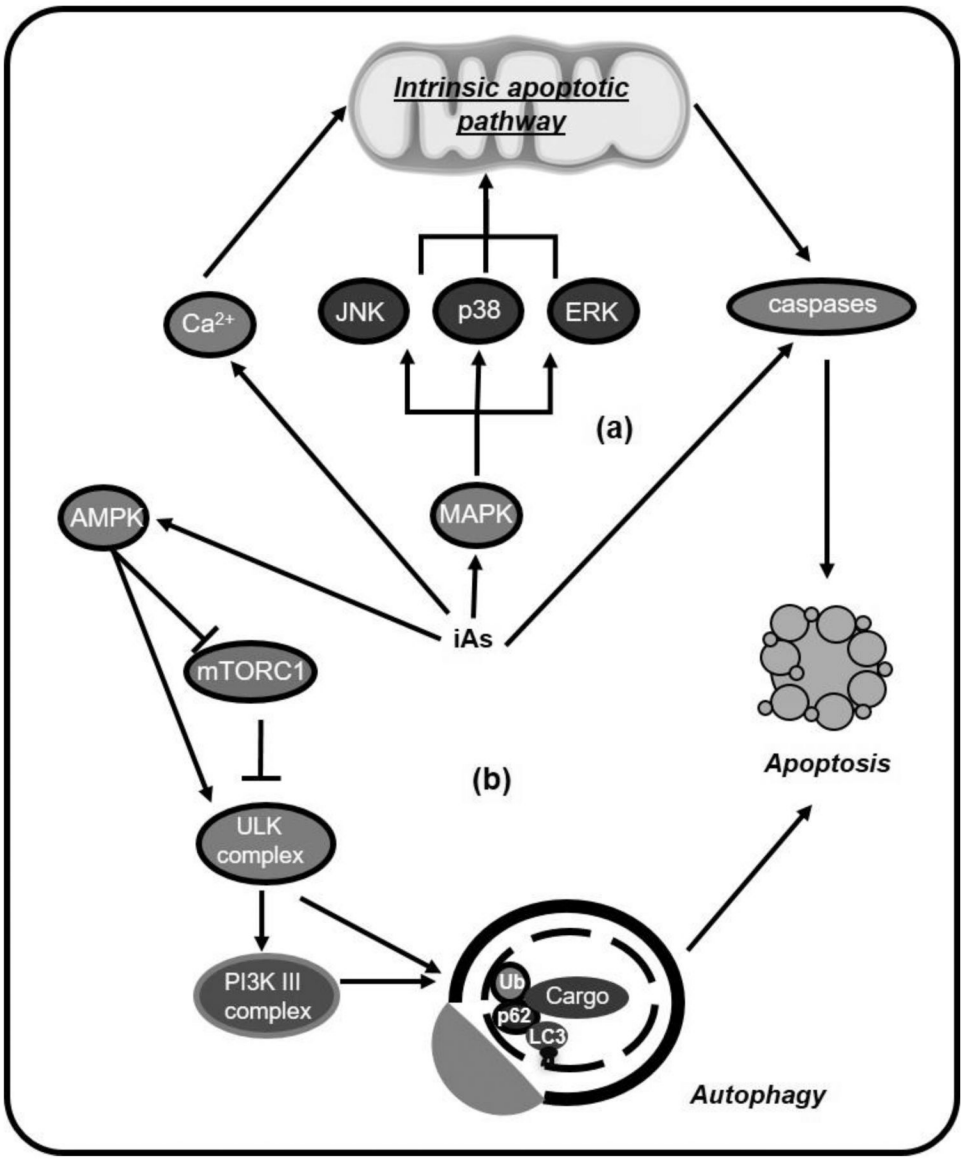

Figure 4. Mechanisms of *iAs*-induced cell death in neuronal cells.

iAs neurotoxicity has been related to the activation of different cell death pathways. **(a)** Exposure to iAs and its methylated metabolites induces caspase-dependent apoptosis, involving the activation of the mitogen-activated protein kinase (MAPK) pathways, specifically c-Jun N-terminal kinase (JNK), p38 and extracellular-signal-regulated kinase (ERK) which are associated with the intrinsic mitochondrial apoptotic pathway. *iAs* also triggers intracellular calcium (Ca^{2+}) increase that mediates apoptosis. **(b)** *iAs*-induced cell death is regulated by autophagy activation through the activation of the AMP-dependent protein kinase (AMPK) and inhibition of the mechanistic (or mammalian) target of rapamycin (mTOR).

Table 1

Toxic effects of Arsenic in the Nervous System

Human studies	Exposure [iAs]	Symptoms / Effects	Ref
Adults $\bar{x}$ 37.6 y (Bangladesh)	Chronic 14.1 ± 3.27 y < 129 μg/L, 130-264 μg/L and > 265 μg/L	⇓ Activity of the plasma cholinesterase enzyme with increasing levels of *iAs* exposure. This enzyme is involved in both liver and brain function and neurotoxicity	[101]
Adults $\bar{x}$ 40 y (India)	Cross-sectional study (5 y) Decrease in the exposure from 190.1 to 37.94 μg/L	⇑ Neuropathy, conjunctivitis and respiratory distress	[17]
Adults $\bar{x}$ 44 years	Single dose 0.25-20 mg/L	Peripheral neuropathy ⇓ motor conduction velocity Marked abnormalities of sensory nerve action potentials	[20]
Adults $\bar{x}$ 43 years (India)	Chronic 128.95 ± 65.54 μg/L	Peripheral neuropathy ⇑ Senescence associated miRNAs and PMP22, a specific target of miR-29a	[102]
Adults (Myanmar)	Subchronic (7 m) ≥ 10 mg/L 10-50 mg/L < 50 mg/L	Weakness and chronic numbness or pain Peripheral nerve disturbances	[18]
Adults 20 - 50 y	Cross-sectional study (2 y) Decrease in the exposure from 115 to 0.025 μg/L	⇑ Subclinical sensory neuropathy (elevated toe vibration threshold).	[19]
Children 8-11 y (Bangladesh)	Chronic 10 μg/L	⇓ Motor function (fine manual control and body coordination)	[11]
Children $\bar{x}$ 7.61 years (Mexico)	Chronic 62.9 ± 0.03 μg As / g creatinine	⇓ Verbal IQ, verbal comprehension and long term memory	[8]
Children $\bar{x}$ 7 years (Mexico)	Chronic > 50 μg/L	⇓ visual/spatial, verbal and motor abilities	[9]
Children $\bar{x}$ 9.67 years (USA)	Chronic (7.3 y) > 5 μg/L	⇓ Full scale IQ, perceptual reasoning, working memory and verbal comprehension	[10]
Mice	**Exposure [iAs]**	**Symptoms / Effects**	**Ref**
C3H	Gestational 85 mg/L	60 weeks of age: Behavioral inflexibility, abnormal formation and disarrangement of the prelimbic cortex	[14]
CD-1	Gestational 20 mg/L	PND 1 (whole brain): ⇑ GSSG, xCT, EAAT3, LAT1 PND 15 (hippocampus): ⇑ GSH, xCT, EAAT3; ⇓ GLT1, NMDA (NR2A-B) / AMPA receptors PND 15 (cortex): ⇑ xCT, EAAT3; ⇓ NR2A PND 90 (hippocampus): ⇓ xCT, NR2B, GluA1, GluA2 PND 15, 90 : ⇓ spatial memory (LTP)	[12, 13]
C57BL/6	Gestational 100 mg/L	PND 21 (striatum): ⇓ NMDA receptors, VGLUT2 and mGluR2	[103]
Swiss Webster	Chronic (1 m) → geriatric age 0.10 mg/L	Striatum and cortex: ⇑ protein ubiquitination, LC3 levels, tyrosine hydroxylase and synuclein accumulation	[31]
3xTgAD model	Gestational → 6 m 3 mg/L	Behavioral impairment Hippocampus: ⇓ ATP and complex I; ⇑ oxidant state Cortex: ⇑ antioxidant response Frontal cortex and hippocampus: ⇑ amyloid β and phosphorylated tau	[104]
Rats	**Exposure [iAs]**	**Symptoms / Effects**	**Ref**
Wistar	Gestational → 4 m 3 mg/L	Behavioral deficits Whole brain : ⇑ AD biomarkers (RAGE, Aβ and BACE1 activity)	[26]
	Acute (9 h) 15 and 20 mg/kg	Disappearance of neurofilament and fibroblast proteins Changes in cytoskeletal composition	[21]

Human studies	Exposure [iAs]	Symptoms / Effects	Ref
	Subchronic (30 d) 10 mg/kg	Sural nerves of the right hind limb: ⇑ Lipid peroxidation, slower nerve conduction velocity; ⇓ conduction area, myelin thickness, area and perimeter of axons	[23]
Experimental Model	**Exposure [iAs]**	**Symptoms / Effects**	**Ref**
Zebrafish (*Danio rerio*)	Subchronic (96 h) 0.05, 5, 15 mg/L	⇑ Locomotor activity and Anxiety Whole brain : ⇓ ATP, ADP and AMP hydrolysis (ectonucleotidases activities)	[105]
	Chronic (90 d) 50 μg/L	Whole brain: ⇓ GSH; ⇑ antioxidant response (Nrf2, HO1, NQO1, Sod, Cox1, GPx1, Cat); ⇑ apoptosis	[106]
	Subchronic (96 h) 1, 10, 100 μg/L	Impaired long term memory and learning ⇑ protein oxidation	[107]
	Larval, juvenile, and adult stage (4 h → 150 days postfertilization) 50, 500 μg/L	Impaired motor function, associative learning and sensorimotor response.	[108]
Nematode (*Caenorhabditis elegans*)	100 μM	Deficits in the structure of sensory neurons ⇓ Locomotor activity,⇑ ROS production	[109]

Abbreviations: AD, Alzheimer's disease; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; APP, amyloid precursor protein; BACE-1, β-secretase; Cat, catalase; COX, cytochrome c oxidase; d, days; γ-GCS, gamma-glutamylcysteine synthetase; GPx1, glutathione peroxidase 1; h, hours; HO1; heme oxygenase1, iAs: inorganic arsenic; IQ, intelligence quotient; ; IL, interleukin; m, months of age; NMDA, glutamate/N-methyl-D-aspartate; NQO1, NADPH dehydrogenase quinone 1; Nrf2, nuclear factor (erythroid-derived 2)-like 2; p62, autophagic receptor sequestosome-1, p62/SQSTM1; PMP22, peripheral myelin protein 22; PND, postnatal day; RAGE, advanced glycation end products; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha; xCT, cystine/glutamate exchanger system; y, years of age.