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Review

Arsenic toxicity in mammals and aquatic animals: A comparative biochemical approach

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ABSTRACT

Arsenic (As) is a widespread pollutant in the world and its toxicity is related to its chemical form, with inorganic forms being considered more toxic than the organic form, and huge differences in effects and processes of metabolism. This paper reviews the potential biochemical mechanisms of uptake of arsenic by aquaporins, capacity for metabolism and cellular efflux of As. It is known that As can affect signaling pathways since it can activate proteins such as ERK2, p38 and JNK, as shown in mammals. A comparison between phosphorylation sites of these proteins is presented in order to determine whether the same effect triggered by As in mammals might be observed in aquatic animals. The toxicity resulting from As exposure is considered to be linked to an imbalance between pro-oxidant and antioxidant homeostasis that results in oxidative stress. So, present review analyzes examples of oxidative stress generation by arsenic. Biotransformation of As is a process where firstly the arsenate is converted into arsenite and then transformed into mono-, di-, and trimethylated products. In the methylation process, the role of the omega isoform of glutathione-S-transferase (GST) is discussed. In addition, a phylogenetic tree was constructed for aquaporin proteins of different species, including aquatic animals, taking into account their importance in trivalent arsenic uptake.

Contents

1. Introduction

Arsenic (As) is a widespread pollutant in various regions of the world (Flora et al., 2005). Arsenic and its compounds are mobile in the environment. Weathering of rocks converts arsenic sulfides to arsenic trioxide, which enters the arsenic cycle as dust or by dissolution in rain, rivers, or groundwater (Mandal and Suzuki, 2002). Chronic exposure to inorganic arsenic can lead to cancer of the skin, lungs, bladder, and liver (Aposhian et al., 2003). This metalloid is commonly found in several chemical forms with different toxicity; thus, inorganic forms of arsenic (arsenite and arsenate) are more toxic, while methylated forms (methylarsonate, MMA and dimethylarsinate, DMA) are considered only moderately toxic (Geiszinger et al., 2002; Fattorini and Regoli, 2004). Other arsenic species, like trimethyl-arsine oxide (TMAO) and tetra-methyl-arsonium (TETRA) are also considered moderately toxic, whereas arsenobetaine (AsB), arsenocholine (AsC) and other arsenosugars (AsS) show no toxicity (Fattorini et al., 2006).

The toxicity resulting from As exposure is considered to be linked to an imbalance between pro-oxidant and antioxidant homeostasis that results in oxidative stress (Shila et al., 2005). However, there are many

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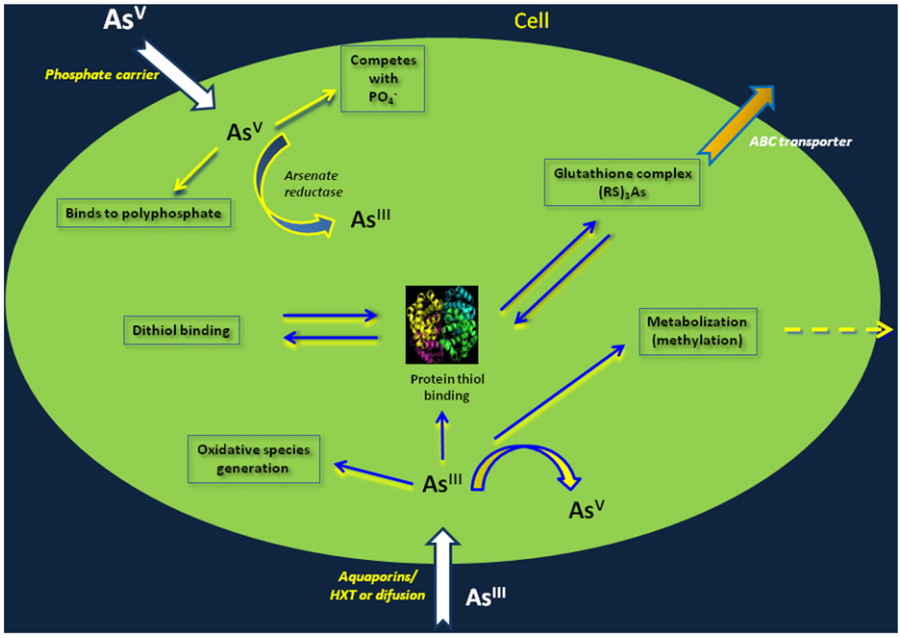

Fig. 1. Toxicological pathways of arsenate (As^V) and arsenite (As^{III}) including cell entry, metabolism, biochemical effects and elimination. Aquaporins and the hexose permease transporter (HXT) are involved in As^{III} entry, whereas phosphate carriers allow the entry of As^V. ABC transporter refers to proteins of the ATP-binding cassette family, associated with the efflux of toxic compounds and/or their metabolites.

differences between arsenite and arsenate effects (Tseng, 2004; Fig. 1). Because of its similar biochemical properties to phosphate, arsenate can replace the former in energy transfer phosphorylation reactions, resulting in the impairment of ATP synthesis (Fattorini and Regoli, 2004). On the other hand, arsenite reacts with sulfydryl groups and can induce structural modification in proteins (Wang et al., 2004), leading to the inactivation of many enzymes (Akter et al., 2005). For example, inhibition of pyruvate dehydrogenase can impair ATP production by blocking the citric acid cycle, which is critical for providing reducing equivalents to the mitochondria that are needed for electron transport (Tseng, 2004).

In aquatic animals, there is evidence for: (1) increases in cytotoxicity in fish cell lines when exposed to sodium arsenite (Wang et al., 2004; Seok et al., 2007); (2) oxidative stress and a decrease of antioxidant enzymes activity, as observed in the polychaete *Laoenereis acuta* and in the fish *Clarias batrachus*, after exposure to As_2O_3 (Bhattacharya and Bhattacharya, 2007; Ventura-Lima et al., 2007); (3) an increase in the antioxidant responses in gills of zebrafish *Danio rerio* (Cyprinidae) exposed to arsenate for two days (Ventura-Lima et al., 2009a); (4) oxidative stress and modulation of the antioxidant system in liver of goldfish (*Carassius auratus*) exposed to arsenite (Bagnyukova et al., 2007); and (5) alterations in the antioxidant system in different tissues of common carp (*Cyprinus carpio*) after exposure to both arsenite and arsenate (Ventura-Lima et al., 2009b).

Taking into account the studies cited above, the objective of the present review is to analyze and to update information about arsenic toxicity in aquatic animals considering mostly biochemical responses that directly or indirectly modulate arsenic metabolism. Due to the scarcity of studies conducted with aquatic animals, some mechanistic steps are based on data obtained from mammals and models using cell lines, as well as by inference after alignment of mammalian protein sequences with the well-known aquatic model zebrafish.

2. Arsenic metabolism

Arsenic toxicity varies widely with its oxidation states. Inorganic species generally are more toxic than organic ones, and arsenite (As^{III}) is about 60 times more toxic than arsenate (As^V). This last species, in turn, is about 70 times more toxic than methylated species as monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA), with the last two forms being considered only moderately toxic (Akter et al., 2005).

In drinking water, As is normally found as As^V, but upon consumption by humans and other organisms it rapidly undergoes metabolic conversion known as biotransformation (Sakurai et al., 2005). Biotransformation of As is a process where first the arsenate is converted into arsenite and then transformed into mono-, di-, and trimethylated products (Thomas et al., 2004), as indicated in the following reactions:

$$As^{III}O_3^{3-} + CH_3^+ \rightarrow CH_3As^VO_2^{3-} + 2e^-$$
$$\rightarrow CH_3As^{III}O_2^{2-} + CH_3^+ (CH_3)_2As^VO_2^- + 2e^-$$
$$\rightarrow (CH_3)_2As^{III}O_2^- + CH_3^+ \rightarrow (CH_3)_3As^VO^-$$

These reactions point to the important fact that As methylation is associated with reduction of As^V to As^{III} (Thomas et al., 2004). Some authors have suggested that the antioxidant glutathione (GSH) plays an important role in arsenic biotransformation as a reducing agent (Kobayashi et al., 2005). The reduction of As to trivalency by GSH has been shown to be linked to the formation of arsenotriglutathione ($As^{III}(GS)_3$), a complex in which As^{III} is bound to the thiol moieties of the cysteinyl residues of three GSH molecules (Thomas et al., 2001). Aposhian et al. (2004) showed that reduction of arsenate to arsenite is catalyzed by an arsenate reductase enzyme that requires the presence of inosine and a thiol compound, possessing a greater affinity for dihydrolipoic acid than GSH.

In mammals, after reduction, arsenite is methylated by arsenite methyltransferase (Akter et al., 2005). Results from *in vitro* assay systems containing rat liver cytosol, arsenite and methylarsonous diiodide ($CH_3As^{III}I_2$) showed that arsenite was the preferred substrate for the methylation reaction, with the conversion of arsenite to methylated metabolites being faster than for arsenate (Thomas et al., 2001). In order for arsenic methylation to occur, a donor of methyl groups must be available. In both *in vitro* and

in vivo studies with mammals, S-adenosylmethionine (AdoMet) has been identified as the methyl group donor (Thomas et al., 2004). The enzyme methylarsonate reductase catalyzes the reduction of monomethylarsonate (MMA^V), dimethylarsonate (DMA^V) and arsenate (As^V) to monomethylarsonous acid (MMA^{III}), dimethylarsonous acid (DMA^{III}) and arsenite (As^{III}), respectively, and the activity of this enzyme is the rate-limiting step for inorganic arsenic methylation (Zakharyan et al., 1999). Methylarsonate reductase has an absolute requirement for GSH, being recognized as the omega isoform of the enzyme glutathione-S-transferase (GST) (Aposhian et al., 2004).

Enzymes of the glutathione-S-transferase (GST) family are multifunctional molecules, which play a key role in cellular detoxification, protecting cells against pollutants or toxicants by conjugating them to glutathione and a variety of endogenous and exogenous electrophilic compounds (Townsend and Tew, 2003). The GST superfamily has been divided into eight distinct classes known as alpha, mu, pi, theta, zeta, sigma and omega (Sampayo-Reyes and Zakharyan, 2006), with this last being distinguished from other GST family members by having a cysteine residue in its active site (Zakharyan et al., 1999). Human GST omega1-1 (hGSTO1-1) is identical to human monomethylarsonic acid reductase (MMA^V), which, as mentioned above, is the rate-limiting enzyme for biotransformation of inorganic arsenic (Sampayo-Reyes and Zakharyan, 2006).

If the methylation process of arsenic is linked to methylarsonate reductase, the omega isoform of GST (GST Ω), it is interesting to analyze the existence of GST omega genes in aquatic animals. The GST Ω gene was identified in the fish species *D. rerio*, *Tetraodon nigroviridis*, *Takifugu rubripes*, *Anoplopoma fimbria* and *Oncorhynchus mykiss* through NCBI Blast Searches of GenBank and a Blast Search in UniProtKB, using the *Rattus norvegicus* (Q9Z339—omega 1 and Q6AXV9—omega 2) and *Xenopus tropicalis* (NP_001005086 and NP_001011256) proteins as queries. The obtained zebrafish sequences (NP_001002621 and NP_001007373) were also compared with the protein database at the Zebrafish Information Network (ZFIN) (University of Oregon, Eugene, OR 97403-5274; World Wide Web URL: http://zfin.org) where their identities were confirmed (ZDB-GENE-040718-365 and ZDB-GENE-041114-67, respectively). The searches resulted in one GST Ω *T. nigroviridis* sequence (CAG05035), one GST Ω *T. rubripes* sequence (AAL08414), one GST Ω *A. fimbria* sequence (C3KH95) and one GST Ω *O. mykiss* (C1BFQ9) sequence. Recently, the omega class glutathione-S-transferase (GST Ω) gene from the polychaete *Neanthes succinea* was cloned and characterized (Jae-Sung et al., 2007). The *N. succinea* protein (ABR24228) sequence was used as a query in order to identify GST Ω sequences among polychaetes. At present, no other GST Ω sequences from polychaetes have been found on the GenBank or UniProtKB databases with this adopted strategy. GST Ω sequences for the gastropod disc abalone (*Haliotis discus*—B6RAZ9), the anemone *Nematostella vectensis* (A7RUN2), the crustacean *Tigriopus japonicus* (B3VHS2-1), the tunicate *Halocynthia roretzi* (Q5NTL) and the bivalve *Crassostrea gigas* (Q5K4L8) were also identified, retrieved from databases and included in the analysis. The alignment was performed using the ClustalX program (Thompson et al., 1997) and a phylogenetic tree was constructed according to the Neighbor-Joining method (Saitou and Nei, 1987) using proportional (*p*) distance with the MEGA 2.1 program (Tamura et al., 2007) (Fig. 2). The amino acid overall mean diversity (Neighbor-Joining) was 53.4% using all sequences and was 26% among fish sequences.

Fattorini and Regoli (2004) showed that the polychaete *Sabella spallanzanii* accumulated As mostly as dimethylated forms, while the estuarine polychaete *L. acuta* accumulated not only mono and/or dimethylated forms but also non-toxic As molecules such as arsenobetaine (AsB) and arsenocholine (AsC) (Ventura-Lima et al., 2007). In other polychaeta species, *Arenicola marina* was found to accumulate As mostly as the inorganic form after arsenate exposure

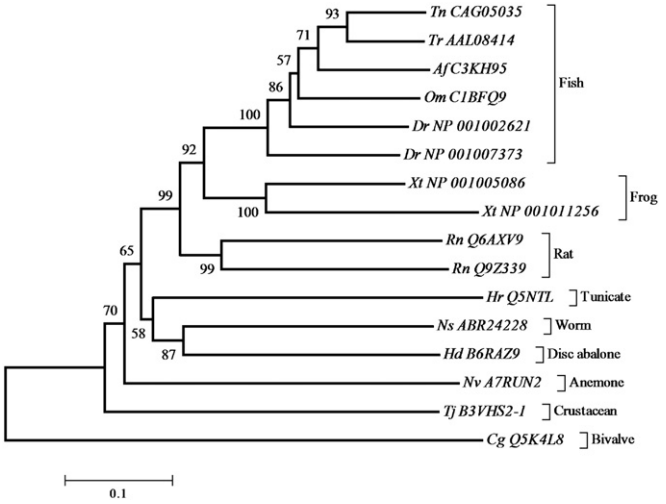

Fig. 2. Phylogenetic analysis of glutathione-S-transferase (GST) omega members. The deduced amino acid sequences were aligned with the ClustalX program and the phylogenetic tree was constructed using the Neighbor-Joining method, proportional (*p*) distance with the MEGA 2.1 program. The phylogenetic tree grouped consistently in one branch (Tn) *Tetraodon nigroviridis*, (Tr) *Takifugu rubripes*, (Af) *Anoplopoma fimbria*, (Om) *Oncorhynchus mykiss*, (Dr) *Danio rerio*, (Xt) *X. tropicalis*, and (Rn) *R. norvegicus*. The second branch with low bootstrap value grouped the polychaete *Neanthes succinea* (Ns) with the tunicate *Halocynthia roretzi* (Hr) and with the disc abalone *Haliotis discus* (Hd). The anemone *Nematostella vectensis* (Nv) and the crustacean *Tigriopus japonicus* (Tj) were placed independently. The bivalve *Crassostrea gigas* (Cg) was a highly divergent member.

(Geiszinger et al., 2002). Taken together, these differences demonstrate that *S. spallanzanii* has the greater capacity to metabolize arsenic while *A. marine* has the lowest, with *L. acuta* showing an intermediate capacity (Monserrat et al., 2007; Ventura-Lima et al., 2007). The predominance of certain arsenic compounds in the tissues might thus be interpreted as a typical feature of a species, and also raises the possibility of different regulation of the GST Ω gene in the polychaeta species cited above. Note that, at present, data regarding the influence of As exposure on GST Ω expression and/or activity are scarce.

In our laboratory we have analyzed the effects of different inorganic As species (arsenite and arsenate) in different organs of the common carp (*C. carpio*), with higher GST Ω activity being observed in gills compared to liver after As (arsenite or arsenate) exposure lasting two days; in fact, while there was measurable As accumulation in the gills, the contrary was true in liver. In gills there was a predominance of monomethylarsenic (MMA) and dimethylarsenic (DMA) (56% and 23%, respectively) while in the liver the low GST Ω activity was paralleled by low As accumulation (Ventura-Lima et al., 2009a). However, authors cannot rule out the possibility of gills symbiotic bacteria contributing for As metabolism, as previously suggested by Company et al. (2004) for mussels living in regions with hydrothermal vent that posses high levels of metals. Maher et al. (1999) showed that most of the organs and blood in the sea mullet (*Mugil cephalus*) contained a large percentage (35–100%) of arsenobetaine (AsB), with the concentration of total arsenic ranging from 0.54 μg/g dry mass in gills to 19.2 μg/g dry mass in liver. Interestingly, Ventura-Lima et al. (2009a) observed in the gills of the common carp a greater accumulation of As than in the liver (ranging from 0.30 to 10.8 and 0.19 to 0.73 μg/g, respectively). The low As levels found in a freshwater fish are in accordance with the report by Fattorini and Regoli (2004), who point out the higher As accumulation in marine animals. Also, authors such as Clowes and Francesconi (2004) considered that the higher capacity for As accumulation in marine animals is modulated by abiotic factors such as salinity. Finally, Ventura-Lima et al. (2009b) detected only inorganic arsenic in the gills

of *D. rerio* (Cypridinae) when exposed during two days to arsenate, suggesting a low capacity to metabolize arsenic, a result that could be related to low GST omega activity, although this needs experimental confirmation. According to authors, the prevalence of As^{III} (instead of As^{V}) in aerobic water could be consequence of microbial activity associated with fish.

In many studies conducted with mammalian models primary emphasis has been placed on the metabolism and clearance of inorganic arsenic (iAs), as reflected by the appearance of this species and its metabolites in urine. DMA^{V} is the last As molecule in the biotransformation pathway in mammals and the methylation of iAs is considered to be a product of the detoxification system (Sakurai et al., 2005). However, recent data point to the fact that trivalent methylated arsenicals, particularly MMA^{III} and DMA^{III} (which are intermediates in the methylation process in mammals) are more active than iAs in terms of enzyme inhibition, cytotoxicity and genotoxicity (Yamanaka et al., 2004). Therefore, the biotransformation can be regarded as a dual process involving both activation and detoxification of As. However, more studies are needed to define whether As metabolism should be regarded as a detoxification or an activation pathway.

Finally it should be mentioned that Mrak et al. (2008) stated that in some organisms other metabolic pathways should lead to the generation of moderately toxic arsenic species like TETRA, TMAO and even non-toxic ones like AsB and AsC.

3. Activation of MAPK family members in response to arsenic exposure

Mitogen-activated protein kinases (MAPKs) are important regulatory proteins through which extracellular signals are transduced into intracellular events. The MAPK family members include ERKs, JNK, p38 and others (Lee et al., 2005). The ERKs, JNK and p38 are activated by a variety of stimuli including cellular stresses, cytokines and growth factors (Humar et al., 2007). However, each one of these kinases is differentially affected by various stimuli; for example, ERKs are most associated with proliferation and responses to growth factors, while JNK and p38 are activated in response to cellular stress and cytokines (Liu et al., 1996; Humar et al., 2007).

It is known that exposure to metals can affect multiple aspects of cellular function including proliferation, apoptosis, differentiation and cell transformation (Harris and Shi, 2003). Many effects are related to the generation of reactive oxygen species (ROS) (Chen et al., 2002), although there is evidence that metals such as arsenic, chromium and nickel can affect cell signaling independently of ROS generation (Harris and Shi, 2003).

In this way, arsenic appears to affect the MAPK pathway. Liu et al. (1996) showed in wild-type PC12 cells that arsenite treatment potently activated both JNK and p38 while ERK was moderately activated. In the same study, the authors observed that co-treatment with the antioxidant N-Acetyl-L-cysteine prevented the activation of the kinases mentioned above, suggesting that an oxidative signal initiates the responses. It is important to remember that arsenite has greater affinity for sulphydril residues, such as those existing in many regulatory proteins, and consequently the structure of these proteins or even the redox state can be modified, initiating signaling pathway events. Oxidative changes to growth factor receptors are likely to be involved in ERK activation by arsenite, while the simultaneous interaction of arsenite with other cell components such as cytokine receptors and/or intracellular proteins may initiate JNK and p38 activation (Liu et al., 1996). These effects of arsenic can alter normal cellular metabolism and cause severe oxidative damage to cell components. Recently, Huang et al. (2009) demonstrated the activation of the ERK signaling pathway in human uroepithelial cells after arsenite treatment and due to this activation the cell was committed to undergo autophagy. In fact, the arsenite appeared to cause autophagic cell death in the malignant cells (Huang et al., 2009).

In GCS-2 cells arsenite also was shown to induce phosphorylation of ERK, JNK and p38 in a dose dependent manner (Habib, 2009). Activation of both mouse JNK and rat ERK2 is correlated with phosphorylation of an amino acid residue at Thr183/Tyr185 (Canagaraj et al., 1997; Wang et al., 2005), while mouse p38 is phosphorylated on residue Thr180/Tyr182 (Wang et al., 2005). Although the activation of MAPKs by arsenite can lead to changes in cell metabolism, few studies have considered the effect of this metalloid on the signaling pathway of other organisms besides mammals.

If the phosphorylation of ERK2, JNK and p38 in other species such as fish and chicken can be induced by arsenite as described for mammals, it would be interesting to analyze the probable phosphorylation residues for these kinases in these species. The identification of protein sequences (ERK2, JNK and p38) from mouse, rat, chicken and zebrafish was performed by an NCBI Blast search. The primary protein sequences employed for analysis were:

(a) *D. rerio* (BAB11813), *Gallus gallus* (AAK56503) and *R. norvegicus* (P63086.3) for ERK2;
(b) *D. rerio* (Q9DGD9), *G. gallus* (XP_421650) and *Mus musculus* (Q91Y86) for JNK;
(c) *D. rerio* (NP_571797), *G. gallus* (XP_001232616) and *M. musculus* (NP_036081) for p38.

The probable phosphorylation sites for threonine and tyrosine residues were identified using NetPhos, version 2.0 (http://www.cbs.dtu.dk) (Blom et al., 1999), a kinase-specific prediction

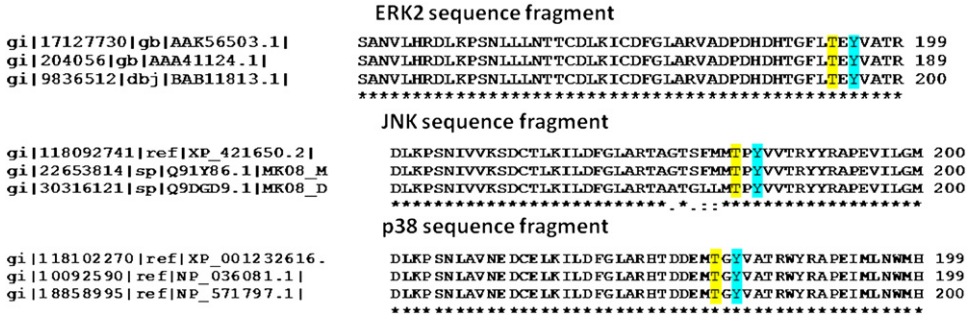

Fig. 3. (a) Alignment of ERK2 primary sequences of the zebrafish *Danio rerio* (BAB11813), chicken *Gallus gallus* (AAK56503) and rat *R. norvegicus* (P63086). (b) Alignment of JNK primary sequences for zebrafish *Danio rerio* (Q9DGD9), chicken *Gallus gallus* (XP_421650) and mouse *Mus musculus* (Q91Y86). (c) Alignment of p38 primary sequence of zebrafish *Danio rerio* (NP_571797), chicken *Gallus gallus* (XP_001232616) and mouse *Mus musculus* (NP_036081). In all cases only fragments where threonine (yellow) and tyrosine (light blue) residues were analytically determined to be phosphorylated are included. The species sequences where the phosphorylation sites were analytically established are: gi|204056|gb|AAA41124.1|; gi|22653814|sp|Q91Y86.1|MK08_M; and gi|10092590|ref|NP_036081.1|. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

tool for protein phosphorylation sites. For ERK2 the software predicted Thr194/Thy196 in *D. rerio*, Thr193/Thy195 in *G. gallus* and Thr183/Thy185 in *R. norvegicus*, this last pair being the same as that described experimentally by Wang et al. (2005) in rat.

For JNK in *D. rerio*, *G. gallus* and *M. musculus* the predicted phosphorylated residues were the same: Thr183/Thy185. Again, NetPhos predicted the same amino acid residues in mouse as those determined analytically by Canagaraj et al. (1997).

Finally, for p38 the predicted phosphorylation sites were Thr191/193Thy for *D. rerio*. For *G. gallus* and *M. musculus* the residues were 180Thr/182 Tyr, the same as those determined analytically by Wang et al. (2005) for mouse.

In order to obtain information and allow inferences about the effects of arsenic upon cell signaling, the sequences of the different species were aligned using the on-line ClustalW2 program (http://www.ebi.ac.uk/Tools/clustalw2/; Larkin et al., 2007). The alignment of these sequences and the phosphorylation residues are depicted in Fig. 3, where it can be seen that the residues are perfectly lined up. Therefore, we can infer that the same effects observed in mammalian species for ERK2, JNK and p38 activation after arsenic treatment might also be observed in other organisms such as fishes and chicken.

4. Influx and efflux of inorganic arsenic species in biological systems

As stated in the Introduction to this review, As^{III} is chemically more reactive than As^{V}; in addition, it can enter the cell by diffusion and, as recent evidence suggests, through the involvement of other transport systems such as aquaporins. The entry of As^{V} to the cells has been linked to phosphate transporters (Thomas, 2007).

Aquaporins (AQP) are transmembrane proteins which facilitate movement of uncharged solutes down a concentration gradient. The expression of AQP3, AQP7 and AQP9 was shown to increase the uptake of As^{III} in yeast and *Xenopus laevis* oocytes (Thomas, 2007), as well as in mammalian cells (Agre and Kozono, 2003; Bhattacharjee et al., 2004; Liu et al., 2004; Lee et al., 2006). In addition, taking into account that AQP7 and AQP10 are sister groups, it is important to analyze genes encoding AQPs 3, 7, 9 and 10 in fishes. The genes coding for AQPs 3, 7, 9 and 10 in fishes were identified using the same strategy used to identify GST omega genes. The well-known amino acid sequences from human (NP_004916), mouse (NP_057898) and frog (NP_001087946) AQP3s, from human (NP_001161), mouse (NP_031499) and frog (NP_001015726) AQP7s, from human (NP_066190) and mouse (NP_071309) AQP9s and from human (NP_536354) AQP10 were used as queries. Searches in the GenBank and UniProtKB databases allowed the identification of two putative AQP3 paralogs in *D. rerio* (NP_998633 and XP_696449), one ortholog in *T. nigroviridis* (CAF91375) and one in *O. mykiss* (Q6L889-1); one AQP7 ortholog in *D. rerio* (NP_956204), *T. nigroviridis* (CAG01413) and *A fimbria* (C3KI76-1); one AQP9 ortholog in *D. rerio* (NP_001028268) and *T. nigroviridis* (Q4S1C7-1) and one AQP10 ortholog in *D. rerio* (NP_001002349) and *T. nigroviridis* (CAG11881). The phylogenetic tree constructed according to the Neighbor-Joining method using proportional (p) distance resulted in four well-resolved terminal clades, thus confirming the presence of AQP orthologues in fishes (Fig. 4). The amino acid overall mean diversity (Neighbor-Joining) was 44.7% using all sequences.

The hexose permease transporter (HXT) is another protein which modulates the uptake of As^{III} by cells, with a higher efficiency compared to AQP (Thomas, 2007). In fact, the polymerization of three $As^{III}(OH)3$ molecules results in something that resembles the structure of hexoses typically transported by HXT. The AQPs and HXT have different tissue distribution patterns and

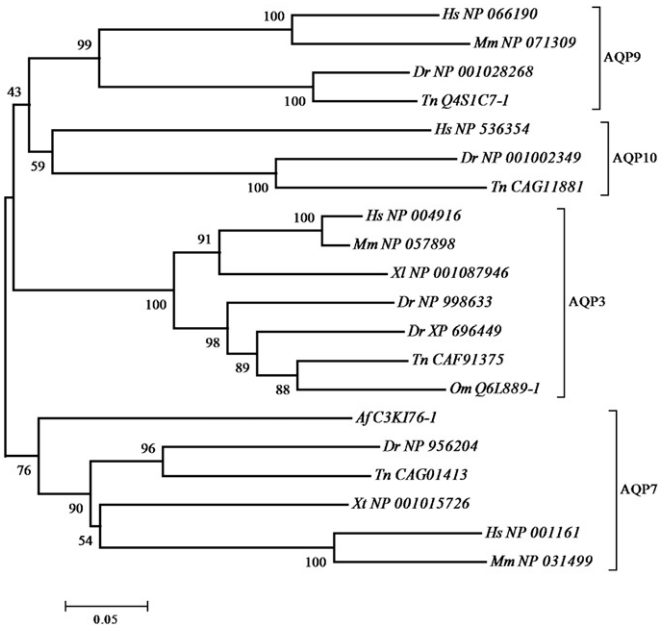

Fig. 4. Phylogenetic analysis of Aquaporins 3, 7, 9 and 10 (AQPs). The deduced amino acid sequences were aligned with the ClustalX program and the phylogenetic tree was constructed using the Neighbor-Joining method, proportional (p) distance with the MEGA 2.1 program. The phylogenic tree identified four well-resolved terminal clades corresponding to each one of the AQPs analyzed. The AQP3 clade grouped consistently *H. sapiens* (Hs), *M. musculus* (Mm), *X. leaves* (Xl), *D. rerio* (Dr), *T. nigroviridis* (Tn) and *O. mykiss* (Om). The APQ7 clade grouped *H. sapiens* (Hs), *M. musculus* (Mm), *X. tropicalis* (Xt), *D. rerio* (Dr), *T. nigroviridis* (Tn) and *A. fimbria* (Af). The APQ9 clade grouped *H. sapiens* (Hs), *M. musculus* (Mm), *D. rerio* (Dr) and *T. nigroviridis* (Tn), whereas the AQP10 clade grouped *H. sapiens* (Hs), *D. rerio* (Dr) and *T. nigroviridis* (Tn).

probably both of these pathways play a significant role in the uptake of arsenic compounds by cells.

Accumulation of arsenite is also influenced by the direct excretion of this molecule mediated by membrane-bound efflux transporters, the multidrug-resistance proteins (MRP types 1 and 2), which are members of the ATP-binding cassette (ABC) family (Choudhury and Klaassen, 2006). In the rat cell line TRL-125, resistance to acute cytotoxic effects of As^{III} was associated with increased expression of MRP1 and MRP2 (Liu et al., 2001). Induction of MRP and the concomitant reduction of aquaporins were responsible for the lower intracellular accumulation of arsenic in a lung adenocarcinoma cell line resistant to As^{III}, which exhibited a decreased influx and increased efflux of arsenic (Lee et al., 2005). It appears that GSH is involved in the function of the ABC transporter, through complexation with As^{III} resulting in arsenic triglutathione ($As^{III}(GS)_3$), which is excreted from cells. A study performed with CHO cells resistant to acute cytotoxic effects of As^{III} demonstrated that the inhibition of GST activity abolished an arsenic-resistant phenotype and that overexpression of GST pi (GSTP) reduced accumulation of arsenic in cells (Thomas, 2007). However, the catalytic role of GSTP in the formation of a complex between trivalent arsenic and GSH has not been demonstrated.

5. Arsenic and oxidative stress

Oxidative stress is one of the theories postulated for arsenic carcinogenesis. Arsenic can stimulate production of reactive oxygen species (ROS), namely the superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$) and peroxyl radical ($ROO^{\bullet}$) (Pi et al., 2003). Sakurai et al. (2005) observed an increase in the production of ROS in TRL 1215 cells after exposure to arsenic (as monomethylarsonic acid; MMA^{V}) and Bhattacharya and Bhattacharya (2007) showed

a peak in the production of hydrogen peroxide (H_2O_2) after *in vivo* arsenic exposure in the fish *C. batrachus*. Oxidative deterioration of polyunsaturated fatty acids through lipid peroxidation (LPO) has been widely accepted as a general mechanism of action for cellular injury (Halliwell and Gutteridge, 1999) and is one of the toxic effects of arsenic (Ramanathan et al., 2003). Induction of LPO and ROS generation by arsenic are considered the main causes of genotoxicity in experimentally exposed rats (Flora et al., 2005), and authors such as Nandi et al. (2005) have observed an increase in the extent of lipid peroxidation in organs including the liver and kidney of rats after arsenic exposure. Arsenite also can induce oxidative damage in proteins due to the high affinity of this metalloid for the sulphydryl groups of proteins (Tseng, 2004). Oxidative damage to proteins is reflected by an increase in the levels of protein carbonyl groups; furthermore, aldehydes, such as 4-hydroxy-2-nonenal or malondialdehyde produced during lipid peroxidation can be incorporated into proteins by reaction with a lysine moiety or the sulphydryl group of cysteine residues to form carbonyl derivatives (Shila et al., 2005). In fact, a rise in oxidized proteins was observed in rat brain after arsenic exposure (Shila et al., 2005), and likewise in the brain of zebrafish (*D. rerio*) (Castro et al., 2009).

The antioxidant and free radical scavenger glutathione (GSH) is important in the preservation of cellular redox status and defenses against ROS and xenobiotics (White et al., 2003). Glutathione and enzymes related to its synthesis comprise a system that maintains the intracellular reducing environment and acts as a primary defense against excessive generation of harmful ROS (Ochi et al., 1994). Glutathione is formed by glutamate, cysteine and glycine and its synthesis depends on the combined activities of two enzymes: glutamate cysteine ligase (GCL) and glutathione synthetase (White et al., 2003). Glutathione appears to play a major role in protecting cells against exposure to arsenic and this statement is supported by studies showing that resistance to arsenic toxicity in mammalian cells (Sakurai et al., 2005) is correlated with higher levels of GSH and higher activities of GSH-related enzymes including GR, GST and GCL (Schuliga et al., 2002). On the other hand, GSH is the substrate for monomethylarsonate reductase which, as mentioned previously, catalyzes the first step in the bioactivation of inorganic arsenic as a human carcinogen (Aposhian et al., 2004). In human keratinocytes an increase was observed in GCL activity and mRNA levels parallel to an increase in the GSH levels after arsenic exposure (Schuliga et al., 2002). Previous studies suggested that GSH offers protection against arsenic toxicity (Sakurai et al., 2005). Treatment of rat liver epithelial cells (TRL 1215) with organic arsenic (monomethylarsonic acid; $MMAs^V$) was not cytotoxic even at concentrations exceeding 10 mM, but it became weakly cytotoxic and induced cell death both by necrosis and apoptosis when GSH was depleted with the GCL inhibitor (*L*-butionine-[*S*,*R*]-sulfoximine; BSO), or the glutathione reductase inhibitor (carmustine) (Sakurai et al., 2004). These data indicate that arsenic toxicity is linked to GSH levels, and when this antioxidant is depleted, arsenic toxicity is increased. These findings suggest that $MMAs^V$ is not highly toxic in mammalian cells, and GSH levels are critical to the eventual toxic effects that methylated species can induce. Oketani et al. (2002) observed that As_2O_3 induced apoptosis in hepatoma-derived cells, and in the same study it was found that the sensitivity of such cells to As_2O_3 was inversely related to their intracellular GSH contents and the intensity of GSH synthesis.

On the other hand, long-term changes to the GSH system resulting from chronic exposure to arsenic may deregulate redox-sensitive cell signaling events, with important consequences for oxidative damage. For example, Perquin et al. (2001) proposed that increased expression of GSH and activity-related enzymes, including GR, improved malignant cell resistance to oxidative stress, favoring cell proliferation and aggressiveness.

As mentioned previously, GSH is thought to participate in the reduction of arsenate (As^V) to arsenite (As^{III}), because depletion of GSH inhibited As^V reduction in isolated cells, such as rat erythrocytes (Csanaky and Grecus, 2005). GSH also acts as a substrate for monomethylarsonate reductase (MMA^V), with this being the rate limiting step for the inorganic arsenic methylation pathway (Aposhian et al., 2004). Therefore, GSH appears to have a double role because it acts primarily as an antioxidant defense and, at the same time, participates in arsenic biotransformation. At present, the role of GSH in arsenic toxicity and biotransformation in aquatic animals remains to be established, although Ventura-Lima et al. (2009b) registered augmented GSH levels in zebrafish gills after As^V exposure. On the other hand, the liver of common carp (*C. carpio*) showed an increase in GSH content after exposure to both arsenite and arsenate while the same was not observed in gills (Ventura-Lima et al., 2009a). This difference between organs may be due to different antioxidant strategies to achieve protection against arsenic toxicity since, for example, the gills displayed augmented G6PDH activity, a response that affords NADPH for the re-generation of reduced glutathione (GSH) from oxidized glutathione (GSSG).

6. Conclusions

It has been established that arsenic toxicity is related to its chemical forms. In fact, some authors have suggested that As is incorporated by cells through AQPs, in mammals and other organisms, after which it can be biotransformed and its metabolites can also exert toxic effects. Thus, the biotransformation of As should be considered also as a bio-activation pathway leading to arsenic toxicity. As can be eliminated by multidrug-resistance proteins after conjugation with GSH; however, studies that address the mechanisms of influx and efflux have been carried out in mammals, and more studies are needed to delineate these mechanisms in aquatic animals.

Although it is known that As can affect signaling pathways mostly in mammals, in this review we compared phosphorylation sites in different mammalian and non-mammalian species. As a result, we can infer that in aquatic animals it is possible that the same effects observed in mammalian models can in fact occur.

Furthermore, it has been shown that arsenic can induce oxidative stress in mammals and also some aquatic animals. However, few studies have been carried out regarding arsenic toxicity and the biotransformation of this metalloid in aquatic organisms such as fish, an issue that deserves more focused research, especially if taking into account that several countries with As pollution problems employ aquaculture practices using pen enclosures (Poersch et al., 2006).

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