Arsenic Induction of Metallothionein and Metallothionein Induction Against Arsenic Cytotoxicity

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Contents

1 Introduction

Several factors contribute to human exposure to Arsenic (As), such as As-compounds in ground water, sodium arsenate (Na-As^{V}) in pesticides, and cigarette smoke. In natural waters, As is mostly found in inorganic forms as oxyanions of trivalent arsenite (As^{III}) or pentavalent arsenate (As^{V}) (Smedley and Kinniburgh 2002). Millions of people are exposed to As concentrations that are above the WHO recommended limit (Garelick et al. 2008; Hashim and Boffetta 2014). In the environment, oxidation states of As vary such as −3, 0, +3 and +5. Organic forms of As are mainly produced by biological activities.

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Depending upon the chemical form, the acute toxicity of arsenicals decreases from inorganic AsIII > inorganic AsV ≫ organic arsenicals (e.g., monomethyl AsV) (Klaassen 1990).

Various tissues and organs are affected by As toxicity, such as the skin, liver, kidneys and lungs. In those tissues/organs, a number of mechanisms have been identified resulting in cytotoxicity (Table 1). At the same time arsenicals were found to be associated with both in vitro and in vivo induced expression of several antioxidant defense systems including glutathione (GHS) and metallothionein (MT) (Table 2). It is important to note that MT is a family of proteins in which 12 members (isoforms) were reported to be actively expressed in mammals (Mehus et al. 2014).

The most important physiological importance of MT, the SH-containing family of low molecular weight proteins, lies in its toxic heavy metal detoxification and essential heavy metal homeostasis (reviewed by Hamer 1986). At the same time MT provides protection against reactive oxygen species (ROS) (Chiaverini and De Ley 2010; Kassim et al. 2013), which is one of the major primary toxic impacts of As exposure (Pi et al. 2002; Kitchin and Ahmad 2003; Valko et al. 2005). Elevated levels of ROS cause oxidative damage within a cell. Such oxidative stress is often directly associated with Zn deficiency (Kloubert and Rink 2015). Zn plays its antioxidant role as a cofactor of the superoxide dismutase (SOD) by modulating the glutathione (GSH) metabolism and MT expression, by competing with iron and copper in the cell membrane and also by inhibiting the nicotinamide adenine dinucleotide phosphate-oxidase enzyme (Cruz et al. 2015). Thus MTs, a major Zn homeostatic group of proteins, participate in controlling intracellular oxidative stress.

Once ingested through drinking water or food, As travels to organs such as the skin, kidneys and the liver through the circulatory system where MTs play an important role in response to toxic levels of As. However, little is known about the association of As toxicity and blood MT. It is expected that the plasma and cells of the circulatory systems including erythrocytes, thrombocytes, lymphocytes and their precursors are well known reservoirs as well as producers of MT (Vandeghinste et al. 2000; Rahman and De Ley 2001; Rahman and De Ley 2008; Maghdooni Bagheri et al. 2011).

The current review highlights the involvement of MT, with more focus on MT synthesis in blood, and in response to As. Finally, we propose how Zn inducible MT might provide protection against As toxicity in blood.

2 Metallothionein Induction and the Role of As

The increase of MT in plasma, bone marrow, erythrocytes, liver, kidneys differs markedly depending on the type and/or the dose of inducer(s). Dietary Zn for example, increases erythrocyte MT more than plasma MT (Grider et al. 1990), again endotoxins induce plasma MT more than the erythrocyte MT (Bremner

Table 1 Mechanism of cytotoxic effect of Arsenic

Effect in cellular physiology/ metabolism	Form of As	Effected/ treated cells/ tissues	(Possible) Impact	Reference
Disruption of oxidative phosphorylation by substituting phosphate in the formation of ATP	As^{V}	–	–	Bhuvaneswaran (1979)
Formation of metal-thiol complex in vicinal Cys of enzymes such as pyruvate dehydrogenase	As^{III}	–	–	Brown et al. (1976) Farrer et al. (2000)
Oxidative DNA damage (ODD)	As^{III}			Kessel et al. (2002)
Increased MMP-2 and MMP-9 activity, Cox-2 expression, and increased rate of cell proliferation of kidney stem cells	As^{III}	Kidney	Kidney cancer	Tokar et al. (2013)
Inhibition of blood δ-aminolevulinic acid dehydratase (ALAD) activity and glutathione (GSH) level	Na-As^{III}	Blood	–	Agrawal et al. (2014)
Increase of ROS and glutathione peroxidase (GPx) activity accompanied by a decreased SOD, CAT and reduced and oxidized glutathione (GSH and GSSG) levels in blood	Na-As^{III}	Blood	–	Agrawal et al. (2014)
Imbalance of pro-/anti-oxidants due to increased ROS thus disruption of the tyrosine kinase Src mediated transcription signalling pathway resulting in transcription of inflammatory cytokines.	As^{III}	Blood	Intensified inflammation	Milnerowicz et al. (2015)
Necrosis with partial apoptosis of macrophages causing release of TNF α at a cytotoxic dose.	As^{III} (5 μM) As^{V} (500 μM)	Macrophage	Carcinogenesis, hepatomegaly, and splenomegaly	Sakurai et al. (1998)

(continued)

Table 1 (continued)

Effect in cellular physiology/ metabolism	Form of As	Effected/ treated cells/ tissues	(Possible) Impact	Reference
ODD is significantly increased in double knock out [MT-1/MT-2] embryonic stem cells compared to the wild type cells	$NaAsO_2$	MT-$1^{-/-}$MT-$2^{-/-}$Embryonic SC	–	Qu and Waalkes (2014)
As induced toxicity in a dose-dependent fashion, by causing fragmentation of DNA, decreased mitochondrial membrane potential, increased intracellular GSH concentration	As^{III}	Rat kidney tubular cell	As-induced apoptosis has been attributed to the intracellular GSH reactive oxidation	Jimi et al. (2004)

Table 2 Expression of MT in response to As

Mode of As exposure	Effect on MT expression	Site of MT expression	Observation/conclusion	Reference
SC As^{III} injection in Rat	↑ MT accumulation ↑ MT-1, 2 mRNA	Liver	As inducible MT expression could be either directly by inducing transcription, or indirectly by post-transcriptional modifications	Albores et al. (1992)
SC injection in Mice As^{III}, As^{V}, MMA^{V}, DMA^{V}	↑ MT accumulation	Liver	Compared to As^{III}, 3-, 50-, and 120-fold higher molar amounts of As^{V}, MMA^{V}, and DMA^{V}, respectively are required for similar increase of hepatic MT content	Kreppel et al. (1993)
SC injection of in Mice As^{III}	↑ MT-1 mRNA	Kidney, spleen, stomach, intestine, heart, lung	MT transcription induction profile by As^{III} is similar to that of Zn or Cd	Kreppel et al. (1993)
As exposure from the environment	↑ MT-3 protein	Human epidermis of squamous cell carcinoma, basal cell carcinoma, and melanoma	High level of MT-3 protein in in cancerous human epidermis of arsenecosis patients correlates As exposure and the skin disorders and related cancers.	Slusser et al. (2014)

(continued)

Table 2 (continued)

Mode of As exposure	Effect on MT expression	Site of MT expression	Observation/conclusion	Reference
In vitro 6-7 μM of As^{III}	↑ MT-1X, 1 F, 2A, 3 mRNA ↓ MT-1A, 1E mRNA	Human astrocytoma (glioblastoma) cell line U87 MG	The increased MT1X, MT1F and MT2A transcription in human glioblastoma cells represent brain tumour acquired resistance to As cytotoxicity while the MT3 increase was suggested to be involved in arsenic-related induction of type II cell death	Falnoga et al. (2012)
In vitro $NaAsO_2$	↑ MT protein ↑ MT mRNA	Embryonic stem cell	As caused concentration-dependent increased expression of MT, and MTF-1	Qu and Waalkes (2014)
In vitro As^{III}	↓ MT protein	Mouse 3 T3 fibroblasts IκB kinaseβ $^{-/-}$	Activation of MKK4-c-Jun NH(2)-terminal kinase pathway, c-Jun phosphorylation, and apoptosis	Peng et al. (2007)
As^{III}	↑ MT-1 mRNA	Mouse *hepa1c1c7* cells	MT1 transcription is induced through MTF1. As ^{III} induction of MT-1 mRNA is lost in MTF1$^{-/-}$ cells. As ^{III} also induces MTF1 binding to the MRE of MT-1	He and Ma (2009)
As^{III} (50 μM) for 24 h	↑ MT protein	5 mM of α-lipoic acid (antioxidant) pretreated HepG2 cells, (for 8 h)	α-Lipoic acid pre-treatment increased MT expression and down-modulates Nrf2 mediated response	Huerta-Olvera et al. (2010)
In vitro As^{III} treatment	↑ MT protein	Rat lung fibroblasts	Dose-dependent disassembly of cellular microtubules, enhanced free tubulin pool and suppression of microtubule associated proteins (MAPs)	Zhao et al. (2012)
Inorganic As^{III} (500 nM)	↑ MT-1 and MT-2 ↑ SOD-1	Kidney stem cells	Transform rat kidney stem cells and partially differentiated progenitor cells to cancer cells	Tokar et al. (2013)

↑ = increase, ↓ = decrease, SC = subcutaneous, As^{III} = arsenite, As^{V} = arsenate, MMA^{V} = monomethylarsenate, and DMA^{V} = dimethylarsenate, MTF-1 = metal-responsive transcription factor-1 MRE = metal response element

et al. 1987). Huber and Cousins (1993) have shown that bone marrow MT expression is highly responsive to the amount of Zn in the diet.

As^{III} enters mammalian cells through multiple routes such as aquaglyceroporins (AQP), organic anion transporting polypeptides (OATP) as well as the glucose permeases namely, GluT1, GluT2 and GluT5 (Porquet and Filella 2007; Reviewed by Maciaszczyk-Dziubinska et al. 2012). Erythrocytes and lymphocytes, the two most abundant cell populations among all the cellular components of the circulatory system, express the highest level of GluT1 (Mueckler et al. 1985; Rathmell et al. 2000). Leukocytes as well as liver, spleen, and testis express AQP9, thus they mediate most As^{III} uptake from blood to liver (Ishibashi et al. 2009). In eukaryotic cells, As^{V} uptake is mediated by the high-affinity phosphate transporter, namely sodium-phosphate cotransporter (NaPiIIb), which is expressed in a variety of cells, such as the brush borders of enterocytes apical pole of alveolar type II cells in the lung, apical membrane of the mammary glands, epididymis cells of the testis, hepatocytes and apical cells of the renal proximal tubule (Murer et al. 2004).

Once As^{III} enters the cytoplasm, it is sequestrated by GSH and transported through ATP-binding cassette (ABC) transporters present at the plasma membranes. In mammals, inorganic As^{III} is methylated, the methylated forms are then exported from the cells by multiple ABC transporters, AQP and glucose permeases (Drobná et al. 2010; McDermott et al. 2010; Carew et al. 2011). In addition, multidrug resistance-associated protein (MRP), also called canalicular multispecific organic anion transporter (MRP1 and MRP2), transports GSH-As^{III} complex (Leslie et al. 2004). MRP2 mediates the efflux of seleno-bis(*S*-glutathionyl) arsinium ion. As^{V} in cytoplasm undergoes a rapid reduction to As^{III} and follows the similar fate of exportation through glucose permeases or MRP1 and 2 (Carew and Leslie 2010).

Major metal MT inducers such as Zn transportation and homeostasis are strictly regulated by Zn binding proteins and Zn transporters (Gaither and Eide 2000) which are different from the As transporters. In circulating erythrocytes, major Zn transporter proteins are ZnT1, Zip8, and Zip10 (Ryu et al. 2008), while in leukocytes they are hZnT-1-9 (Overbeck et al. 2008).

Compared to the extent of research investigating MT expression in relation to As cytotoxicity in different cells and organs (Table 2), studies in the hematopoietic system are scarce. In arsenicosis patients, MT mainly MT-1A and 2A transcripts levels in blood and buccal cells were found positively correlated. When compared to healthy subjects, MT levels are significantly lower in arsenicosis patients (Liu et al. 2007). Inorganic As^{III} resistant multiple myeloma (MM) cells have shown an increased expression of the MT-2A, which was found to chelate intracellular inorganic As^{III} (Zhou et al. 2005). In our laboratory, we have found that in vitro human cord blood mononuclear cells (MNC) expressed MT in response to 50 μM of Na-As^{V} (Fig. 1b), albeit, the level of MT expression was higher when MNCs were treated with 100 μM of Zn (Fig. 1a) (Rahman 2001).

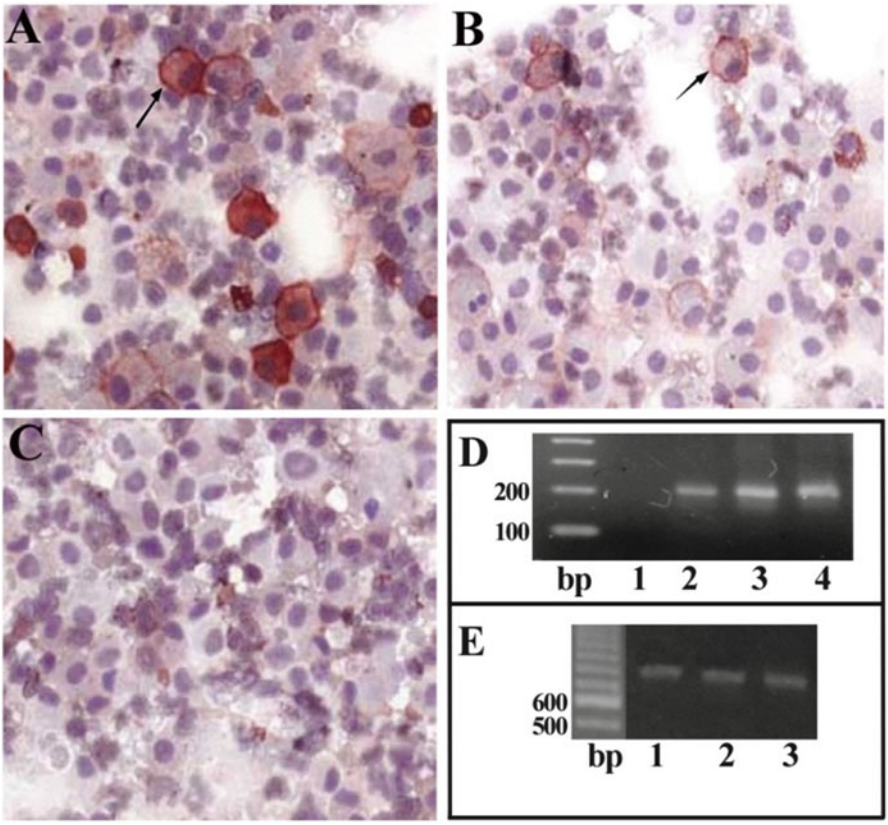

Fig. 1 MT expression in human cord blood MNC in response to in vitro treatment with Na-As^V. MT (*red* at the peri-cytoplasmic spaces) is expressed in MNCs treated with 100 μM of Zn (**a**) and 50 μM of Na-As^V (**b**). Control cultures (**c**), without Zn or As^V, did not show any detectable MT. (**d**) Amplified retro-transcripts of total MT isogenes was similar in MNC cultures treated with 100 μM of Zn (*lane 3*) and 50 μM of As^V (*lane 4*) but higher compared to the control culture (*lane 2*), no band (*lane 1*) was detected after RT-PCR using deionized water instead of mRNA. (**e**) Band intensity for amplified G3PDH retro-transcripts was similar in control (*lane 1*), Na-As^V (*lane 2*) and 100 μM of Zn (*lane 3*) treated cultures. bp, 100 base pair DNA size marker (Rahman 2001)

3 Interaction of As with Blood Proteins

Binding of As to blood proteins such as plasma proteins is a complex phenomenon as it varies from species to species in animals, the route of administration, as well as the proteins involved in the binding. Among different reactive forms of the arsenicals, As^{III} has a high affinity for thiolates of Cys and the imidazolium nitrogen of histidine (His) residues. Typically As^{III} forms three-coordinate trigonal–pyramidal complexes with three Cys in proteins. In hemoglobin (Hb), As^{III} binds with $Cys^{13}\alpha$ and was found responsible for As accumulation in blood (rats fed with As). The

relative reactivity of the Cys in rat Hb was suggested in the decreasing order of: Cys $^{13}\alpha \gg Cys^{111}\alpha > Cys^{104}\alpha$ and $Cys^{13}\alpha \gg Cys^{125}\beta > Cys^{93}\beta$ (Lu et al. 2008). Thus, it is expected that As is bound to Hb through vicinal thiol groups in spleen, bone marrow, plasma and in packed cells.

Besides Hb, As also binds to proteins which have a M_r of 100 kDa, 450 kDa or >2000 kDa in liver cytosol. It has been shown by de novo peptide synthesis that As^{III} -Cys interactions stabilise three-helix bundles found in aqueous solutions (Farrer et al. 2000). When analysing the serum proteins in patients on continuous ambulatory peritoneal dialysis, only the inorganic As species were found to be able to bind to serum proteins, where transferrin is the main carrier (Zhang et al. 1998). It was also shown that, after in vitro incubation in human serum, inorganic As^V binds with serum transferrin (De Kimpe et al. 1993).

Intravenous administration of As^{III}/As^V to mice and rabbits has shown that the percentage of As bound to plasma proteins was 20 % (Vahter and Marafante 1983). When given an intraperitoneal (IP) administration of As^{III} (1 μg of As^{III}/kg mass of the rabbit), the binding of As in plasma proteins increases by about 10 % and 50 % at the 5th and 48th hour (Bertolero et al. 1981). However, prolonged time lapse resulted in gradual decrease of As after IP administration in rabbits. When protein-bound As reached a maximum of 18 % of the total administered As within 20 h, it is then reduced to about 10 % at 120 h (De Kimpe et al. 1996). However, while the percentage of plasma proteins bound As in marmoset monkeys could be as high as 70 % (Vahter et al. 1982), it can also be very low in dogs (Neiger and Osweiler 1989).

4 Interaction of As with Metallothionein

Generally, the two-domain (α and β) MT binds to divalent metals (M) to form two metal–thiolate clusters with stoichiometries of: M_4S_{Cys11} in α-domain and M_3S_{Cys9} in β-domain (Fig. 2a). Using human recombinant MT 1A, Ngu and Stillman (2006) reported that As^{III} binds with stoichiometries of $As_3\ S_{Cys9}$ in both β domain and $As_3\ S_{Cys11}$ in α domain (Fig 2b).

Size exclusion chromatography with inductively coupled plasma mass spectrometry analysis of reaction mixtures between As^{III} and MT clearly demonstrated the formation of complexes of arsenic with MT. Analysis of the complexes using electrospray quadrupole time-of-flight tandem mass spectrometry revealed the detailed binding stoichiometry between As and the 20 Cys residues in the MT molecule (Jiang et al. 2003). Inorganic As^{III} and its two trivalent methylation metabolites, monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}), readily bind with MT (Jiang et al. 2003). Each MT molecule could bind with up to six As^{III}, 10 MMA^{III}, and 20 DMA^{III} molecules, consistent with the coordination chemistry of these arsenicals (Jiang et al. 2003).

The time- and temperature-resolved electrospray ionization mass spectrometry, Ngu et al. (2008) demonstrated that As^{III} binds to MT in a non-cooperative manner

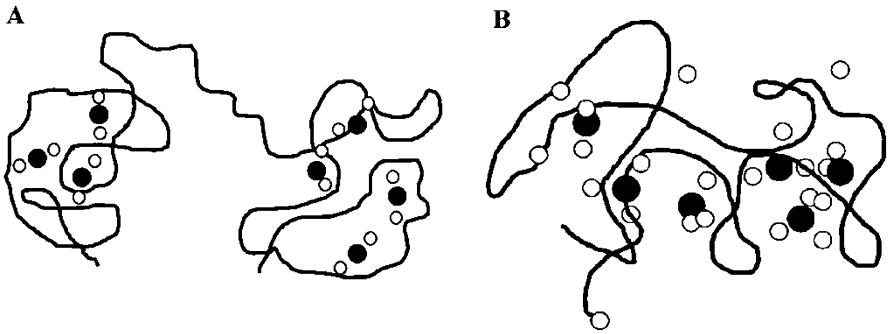

Fig. 2 Metal binding of cysteine (Cys) residues of MT. (**a**) Four divalent metal ions (M^{2+}) such as Cd^{2+} or Zn^{2+} (*filled circles*) generally binds with α-domain while the number of that in β-domain is 3. M^{2+} are bonded with SH (-S-) group of Cys (circles). (**b**) Six As atoms (*filled circles*) are bound to 18 Cys (*white circles*) in MT. Polypeptide backbone is shown in black ribbon with an approximate location of M^{2+}, As and Cys. Figure **a** and **b** are simplified from the corresponding models reported by Ruttkay-Nedecky et al. (2013); and Ngu and Stillman (2006) respectively

involving six sequential reactions in which binding begins with the α-domain followed by the β-domain. Compared to the single domain MT present in cyanobacteria, the two-domain structures allow MT to bind metals faster, and thus make it an efficient metal scavenger (Ngu et al. 2008).

At neutral pH (pH 7), where free As^{III} is not stable, As^{III} that is bound to the recombinant human MT-1A is stable and translocates via protein-protein interactions to other MTs. In vitro studies also confirms that As^{III} transfers from the two-domain β-α-hMT-1A to the isolated apo-β-hMT and apo-α-hMT, where demetallation of the As(6)-βα-hMT occurs in noncooperative manner as apo- and partially-metallated species coexisting in equilibrium conditions (Ngu et al. 2010).

Studies on binding of different metals with MT reveal that mercury (Hg) has the highest affinity for MT while As, Ca and Mo had a limited affinity. When different metals were added to Zn-MT complex, Zn that bound to MT could be replaced in the order of the following affinity: Cd (1.33 μM) > Pb (1.46 μM) > Cu (1.93 μM) > Hg (3.93 μM) > Zn (8.06 μM) > Ag (10.4 μM) > Ni (474 μM) > LCo (880 μM). Al, Cr, Fe, Mg, Mn, Tl and V had no effect on Zn binding even at 1.0 mM (Waalkes et al. 1984). Later Nielson et al. (1985) proposed the metal binding affinity to thiol in the order of: Hg > Cu > Cd > Zn > Ni = Co. Again, Hamer (1986) proposed the affinity in the order of: Hg > Ag > Cu > Cd > Zn.

When partially metallated Cd-MT and Zn-MT were considered the more stable form of metal-apoMT complex, As^{III} transfer at pH 7 is found to be dependent on protein-protein interaction (Ngu et al. 2010). Nonetheless, the cellular redox state as well as the concentration of other biological metal chelators determines the Zn transfer from and to MTs (Jacob et al. 1998). Although initially, Cd or Zn binding to apo-MT is reported to be cooperative (Nielson and Winge 1983), recently, by using recombinant human MT-1A, Sutherland et al. (2012) concluded that the metalation of apo MT occurs in a non-cooperative fashion for both Zn^{2+} and Cd^{2+}.

The binding of As to MT was also reported to be non-cooperative (Ngu et al. 2008). These lines of evidence suggest that even though As toxicity such as increased ROS could induce MT synthesis, the apoprotein might prefer free Zn^{2+} in the cytosol.

Notably, free Zn^{2+} maintains equilibrium in blood through different routes of exchange such as through the erythrocyte membrane permeability. Intracellular Zn^{2+} was found to maintain about 129 μmole/10^{13} erythrocytes while the main component of Zn^{2+} buffering is Hb, with a dissociation constant of about 2×10^{-8} M (Simons 1991).

5 Accumulation and Metabolism of As

Multiple myeloma cells of bone marrow treated with inorganic As^{III} show intracellular biotransformation from As^{III} to As^{V}. Such biological oxidation of As^{III} was described as a protective mechanism of the cell against As cytotoxicity (Falnoga et al. 2007). As^{V} is reduced to As^{III} by CDC25 phosphatases or arsenate reductases (Bhattacharjee et al. 2010). However, biomethylation, particularly the production of As^{III} containing methylated metabolites, is a process that activates As both as a toxin and a carcinogen (Smith et al. 2009). In liver, the intracellular As^{III}-methyltransferase methylates As resulting in formation of both mono and dimethyl As^{V} and As^{III}, and is eventually excreted through bile and urine (Thomas et al. 2007). AQP9 is found to be involved not only in As^{III} uptake from blood to liver, but also in the removal of methylated forms of As down the concentration gradient from hepatocytes to the blood flow to end up in urine (Liu et al. 2006; Carbrey et al. 2009; McDermott et al. 2010). Furthermore, clinical and epidemiological studies have proven that affinity for thiol groups renders As binding to SH moieties of critical proteins like keratin (Lindgren et al. 1982; De Kimpe et al. 1999). Therefore, a variety of skin lesions were linked to As-toxicity (Chen et al. 1988; Brown et al. 1997; Yu et al. 2006).

6 As Cytotoxicity and the Role of Essential Metals

It can be expected that cellular MT induction might act as protective mechanism against As toxicity. This is because production of ROS is one of the major toxic impact of As exposure while MT provides protection against such oxidative stress (Chiaverini and De Ley 2010; Kassim et al. 2013). Induction of MT is achieved through a variety of mechanisms which includes activation of: (1) metal response elements (MRE) by the Zn binding metal-responsive transcription factor (MTF-1) (2) glucocorticoid response elements (GRE) (Kelly et al. 1997), and (3) antioxidant (or electrophile) response element (ARE), in response to the

redox status (Andrews 2000). Zn is also an important regulator of GSH synthesis, where GSH is involved in As excretion (Kala et al. 2000). Zn deficiency is accompanied by the increase in ROS (Kraus et al. 1997; Kojima-Yuasa et al. 2005). In vitro treatment of tARPE-19 cells with 150 μM Zn caused 70 % increase in GSH levels through ARE activated de novo synthesis. ARE activation and GSH synthesis could be inhibited by silencing Nrf2 expression (Ha et al. 2006). Thus, activation of MRE and ARE, by essential nutrients such as Zn might prove beneficial in reducing As toxicity specially to minimize the ROS mediated cytotoxicity.

Cellular metabolism consistently generates ROS, where intracellular GPx plays an important role to reduce ROS such as H_2O_2 to water, hence limiting the harmful effects of the ROS. GPx is a selenocysteine-containing enzyme, expression of which is strictly regulated by the supply of Se and selenocysteine (reviewed by Lubos et al. 2011). Therefore, the in vivo acceptable range of Se supplement to induce antioxidant mechanisms such as GPx and GSH might be beneficial in the reduction of As toxicity, which is mostly linked with ROS. Notably, several lines of evidence have proven the beneficial impact of essential metals such as Zn and Se supplement against metal toxicity, such as cadmium and chromium (Table 3). Cd toxicity on sperm motility and the testicular antioxidant status could be restored by Se and Zn supplement (Saïd et al. 2010). Again Se supplements improved renal toxicity biomarkers' levels and antioxidant enzyme activities in $K_2Cr_2O_7$ administered renal damages (Soudani et al. 2010). Similarly, co-administration of Se with $K_2Cr_2O_7$ restored hematological dysfunction related to the Cr exposure to near-normal values (Soudani et al. 2011). Furthermore, based on a number of in vivo and in vitro studies, McCarty (2012) proposed Zn supplement to ameliorate pathogenic impact of Cd toxicity as Zn is known to have protective anti-inflammatory, antioxidant, and immunosupportive effects. Therefore, it is not unexpected that Zn and Se supplement could be beneficial to minimize cytotoxicity exerted by As (Fig. 3).

7 Conclusion

As, commonly known as a metalloid, has been used to treat cancer, such as the acute promyelocytic leukaemia (Dilda and Hogg 2007), infectious diseases (Frézard and Demicheli 2010) and sleeping sicknesses (Chappuis 2007), the same metalloid is also able to cause cancer and damage the liver and the kidneys. As a means of treatment to As induced damages, essential metals such as Zn and Se could be beneficial, as these metals can induce both intracellular MT and antioxidant mechanisms.

Table 3 Essential metal supplement to treat toxic metal exposure

Toxic metal exposure	Toxic impact	Essential metal supplement	Beneficial impact	Experimental model (Reference)
Cd (Male rat)		Se and Zn	Restoration of the sperm motility and the testicular antioxidant status	In vivo (Rat) (Saïd et al. 2010)
Cd toxicity		Zn	Protective anti-inflammatory, antioxidant, and immunosupportive effects	Review based Hypothesis founded on in vivo and in vitro studies. (McCarty 2012)
$K_2Cr_2O_7$ for 21 days	Renal damages with a significant increase in kidney malondialdehyde, superoxide dismutase, plasma creatinine, and uric acid levels, while catalase, glutathione peroxidase, non-protein thiol, Metallothionein and plasma urea levels decreased	Se	Improved malondialdehyde, renal biomarkers levels and antioxidant enzyme activities. Kidney histological studies confirmed biochemical parameters and the beneficial role of selenium	In vivo (Rat) (Soudani et al. 2010)
$K_2Cr_2O_7$ (700 ppm), in drinking water	Increase of malondialdehyde and protein carbonyl levels and a decrease of sulfhydryl content, glutathione, non-protein thiol, and vitamin C levels. A decrease of enzyme activities like catalase, glutathione peroxidase, and superoxide dismutase activities was also noted in erythrocytes	Se (0.5 mg/kg of diet) for 3 weeks	Co-administration of Se with $K_2Cr_2O_7$ restored the parameters cited above to near-normal values.	In vivo (Rat) (Soudani et al. 2011)

8 Summary

Millions of people are exposed to a toxic level of arsenic (As). Oxidative stress due to As is evident in organs such as the skin, liver, kidneys and lungs. Several intracellular antioxidant defense mechanisms including glutathione (GSH) and metallothionein (MT) have been shown to minimize As cytotoxicity. The current

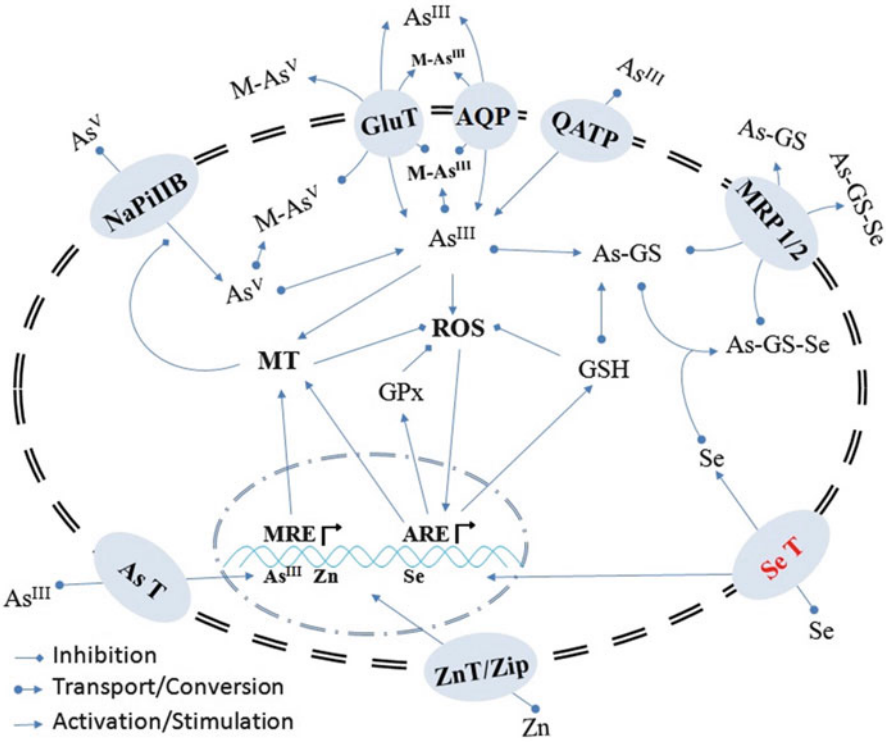

Fig. 3 Intracellular As toxicity and its possible remediation. Once internalized, As^{III} and As^{V} via As^{III} will generate reactive oxygen species (ROS). As^{III} and As^{V} via As^{III} can also induce metallothionein (MT) which in turn will reduce ROS and block intracellular transport of MT respectively. If given Zn and Se supplement, As^{III} inducible ROS can be further reduced by induction of additional MT and glutathione peroxidase (GPx) or glutathione (GSH). [*AQP* aquaglyceroporins, *ARE* antioxidant response elements, *AsT* As transporter, *GluT* glucose permease, *M-As* methylated As, *MRE* metal response elements, *MRP* multidrug resistant protein, *NaPiIIb* sodium-phosphate cotransporter, *QATP* organic anion transporting polypeptides, *ZnT/ZIP* Zn transporter proteins]

review summarizes and the involvement of MT as an intracellular defense mechanism against As cytotoxicity, mostly in blood. Zinc (Zn) and selenium (Se) supplements are also proposed as a possible remediation of As cytotoxicity. In vivo and in vitro studies on As toxicity were reviewed to summarize cytotoxic mechanisms of As. Intracellular antioxidant defense mechanisms of MT are linked in relation to As cytotoxicity. In addition, in vitro potential of pentavalent inorganic As to induce MT biosynthesis was evaluated in human peripheral blood mononuclear cells. Arsenic uses a different route, compared to major metal MT inducers such as Zn, to enter/exit blood cells. However, a number of in vivo and in vitro studies showed that upregulated MT biosynthesis in blood components are related to toxic levels of As. Despite the cysteine residues in MT that aid to bind As, MT is

not the preferred binding protein for As. Nonetheless, intracellular oxidative stress due to As toxicity can be minimized, if not eliminated, by MT. Thus MT induction by essential metals such as Zn and Se supplementation could be beneficial to fight against the global As toxicity.

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