Arsenic: Signal Transduction, Transcription Factor, and Biotransformation Involved in Cellular Response and Toxicity

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Annu. Rev. Pharmacol. Toxicol. 2007. 47:243–62

First published online as a Review in Advance on September 26, 2006

The *Annual Review of Pharmacology and Toxicology* is online at http://pharmtox.annualreviews.org

This article's doi:
10.1146/annurev.pharmtox.47.120505.105144

0362-1642/07/0210-0243$20.00

Key Words

oxidative stress, nitric oxide, electrophile, Nrf2

Abstract

Arsenic is a naturally occurring metalloid that causes oxidative stress. Exposure of humans, experimental animals, and cultured cells to arsenic results in a variety of diverse health effects, dysfunction of critical enzymes, and cell damage. In this context, one area of arsenic study has been the role of its metabolism. Like organic chemicals, arsenic undergoes reduction, methylation, and glutathione conjugation to yield polar metabolites that are substrates for transporters. These events suggest that transcription factor(s) controlling the upregulation of antioxidant proteins, Phase II xenobiotic-metabolizing enzymes, and Phase III transporters should affect arsenic-mediated oxidative stress and the steady-state level of arsenic in the cells. In this review, we summarize recent progress in arsenic toxicity in terms of disrupted signal transduction cascades, the transcription factors involved, and arsenic biotransformation, including a novel pathway.

INTRODUCTION

Arsenic is ubiquitously distributed in nature throughout Earth's crust. This metalloid forms a complex with pyrite, for which it has a strong affinity (1). Under certain conditions (e.g., pH, redox conditions, temperature, solution composition, etc.), however, arsenic is readily dissociated from the complex and enters the groundwater (2). For this reason, the major source of exposure to arsenic for the general population is naturally polluted drinking water from wells. In East Asia, including Bangladesh, West Bengal, India, Vietnam, Thailand, and China, more than 30 million people are chronically exposed to arsenic (1). The arsenic limit set for drinking water in China is 50 μg/l, in spite of the present World Health Organization drinking water guideline value of 10 μg/l, and numerous adverse effects, such as skin lesions, hypertension, Raynaud's phenomenon, ischemic heart disease, peripheral vascular disorders such as blackfoot disease, severe arteriosclerosis, neuropathy, and/or cancer, have been attributed to chronic arsenic exposure (3–7).

A recent topic of arsenic toxicity is the role of biotransformation; methylation of inorganic arsenic species has been thought of as a detoxification process, but methylated arsenites are much more toxic than inorganic arsenic. To elucidate the molecular basis for these manifestations of arsenic toxicity observed in humans, extensive studies with different cultured cells have been conducted over the past decade. The current consensus in studies with cultured cells, experimental animals, and humans is that arsenic is a metalloid that causes oxidative stress through the generation of reactive oxygen species (8–11). Increased reactive oxygen species levels in the body would influence the steady-state level of biomolecules, such as nitric oxide (NO), that react rapidly with reactive oxygen species, such as superoxide, leading to substantial reduction of NO production. Consistent with this notion, exposing humans, rabbits, and rats to arsenic causes decreased NO levels in the body (11–13), thereby diminishing NO-dependent vascular tone (13). On the other hand, reactive oxygen species–triggered protein phosphorylation plays a critical role in cellular signal transduction and disruption of these signal transduction pathways by arsenic is thought to be associated with pathophysiological alterations, resulting in deleterious effects. There is no doubt that arsenic affects signaling cascades, thereby activating (or inactivating) transcription factors that control expression of downstream genes. Some of the gene products upregulated by arsenic exposure act as antioxidant proteins, Phase II xenobiotic-metabolizing enzymes, or Phase III transporters. Indeed, this phenomenon accounts for an adaptive response of the cells to arsenic. In this chapter, we review arsenic-mediated signal transduction pathways associated with pathophysiological alterations and discuss the role of the transcription factor, Nrf2, in the suppression of oxidative stress and cellular accumulation during arsenic exposure.

PRODUCTION OF REACTIVE OXYGEN SPECIES

A major cause of oxidative stress during arsenic exposure seems to be generation of reactive oxygen species. Flavin enzymes such as NAD(P)H oxidase and NO synthase isozymes have been proposed to be involved in the generation of reactive

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

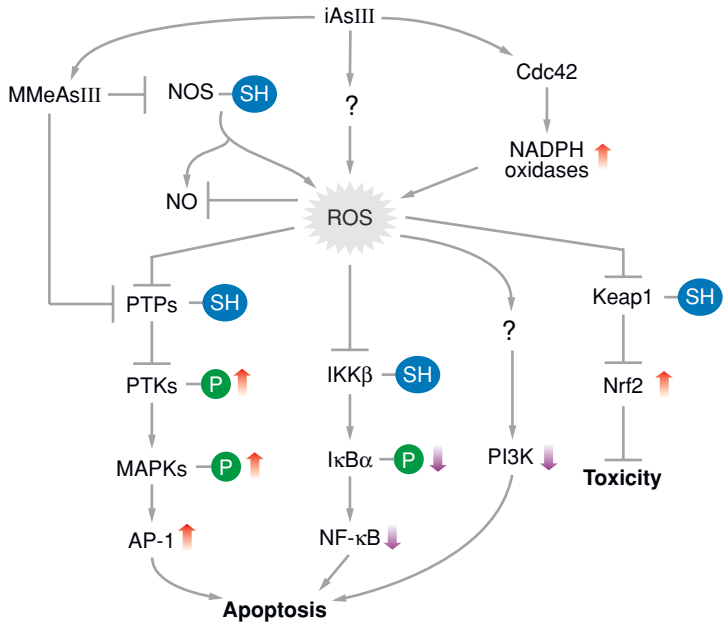

Figure 1

Arsenic-mediated reduction of NO level, signal transduction cascades, and alteration in transcription factors. iAsIII, inorganic arsenite; MMeAsIII, monomethylarsenous acid; NO, nitric oxide; ROS, reactive oxygen species; PTPs, protein tyrosine phosphatases; PTKs, protein tyrosine kinases; MAPKs, mitogen-activated protein kinases; IKK, IκB kinase; PI3K, phosphoinositide 3-kinase; AP-1, activator protein-1; NF-κB, nuclear factor-κB; Nrf2, nuclear factor-erythroid 2-related factor 2; Keap1, Kelch-like ECH-associated protein 1.

oxygen species associated with arsenic exposure. Arsenic upregulates NAD(P)H oxidase at different levels, e.g., gene expression of $p22^{phox}$ (14) and translocation of Rac1 (15) in cultured cells, and thus enhances superoxide production. It has recently been found that arsenic activates NADPH oxidase through cdc42 activation in mouse SVEC4–10 endothelial cells (16) (**Figure 1**). NO synthase isozymes generate high levels of NO, with minimal superoxide from their substrate, L-arginine, under normal conditions. However, uncoupling of NO synthase isozymes, resulting in decreased NO production and concomitant reduction of molecular oxygen to superoxide, does occur under unusual circumstances [e.g., decrease in substrate or cofactor 5,6,7,8-tetrahydrobiopterin (BH_4)] (17, 18). Interestingly, significant reduction of BH_4 levels, but not of L-arginine, is seen following prolonged exposure of rabbits to inorganic arsenate (iAsV) (11). The arsenic-mediated loss of BH_4 could be due to its destruction by superoxide (19), generated through the upregulation of NAD(P)H oxidase.

During an arsenic-induced state of oxidative stress, the reaction of NO with reactive oxygen species should be considered because this metalloid is found to decline systemic NO level in vivo (11–13). NO has a half-life of several seconds in biological systems. Superoxide can react with NO rapidly with a rate constant of $4.3–19 \times 10^9$ M^{-1} S^{-1} (20–22). Therefore, this reactive oxygen species is recognized as a molecule

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

that determines the half-life of NO. Because the reaction between NO and superoxide to form peroxynitrite was shown to be increased in atherosclerosis (23, 24), such a reactive nitrogen species production might be expected during the course of arsenic exposure. Consistent with this notion, peroxynitrite generation, as determined by increased nitrotyrosine production, and exacerbation of atherosclerosis, as evaluated by atherosclerotic plaque formation, have been observed in ApoE and LDL receptor double knockout mice given inorganic arsenite (iAsIII, 10 μg/ml) (25). Overall, it is likely that arsenic is a naturally occurring metalloid that enhances reactive oxygen species production and suppresses NO levels via multiple mechanisms. For example, monomethylarsonous acid (MMeAsIII) produced from iAsIII as described below covalently binds to reactive thiols of endothelial NO synthase, resulting in reduction of its enzyme activity (26) (**Figure 1**). Because oxidative stress and decreased NO bioactivity are known to exacerbate cardiovascular dysfunction, arsenic-mediated vascular disease may occur through these pathways.

DISRUPTION OF SIGNAL TRANSDUCTION

It is well recognized that reactive oxygen species play a critical role in signal transduction pathways and transcription factor regulation (27). Several lines of evidence have indicated that arsenic affects some signal transduction cascades, and thus activates (or inactivates) transcription factors. Epidermal growth factor receptor (EGFR) is one of the protein tyrosine kinases (PTKs), activated in response to EGF binding. EGFR transactivation is negatively regulated by protein tyrosine phosphatases (PTPs). Because PTPs have a preserved thiol group with a pKa value of less than 6, it is among the numerous species involved in dephosphorylation activity (28). Thus, if the thiol groups of PTPs undergo oxidation by reactive oxygen species, transactivation of EGFR without EGF binding occurs because of a transient inactivation of PTPs. Subsequently, activated EGFR causes the phosphorylation of the mitogen-activated protein kinases (MAPKs) signal transduction pathway, which consists of three subgroups: extracellular signal-regulated kinase1/2 (ERK1/2), stress-activated protein kinase/Jun-*N*-terminal protein kinase (SAPK/JNK), and p38 MAPK (29). The ERK1/2 signaling pathway plays an important role in cellular growth and differentiation. The SAPK/JNK signaling pathway and p38 MAPK that are activated by environmental stresses such as UV irradiation, endotoxin, inflammation, oxidative stress, and tumor necrosis factor (TNF) appear to regulate mitochondrial-dependent apoptosis in the proapoptotic direction (30, 31). The activated SAPK/JNK can translocate to the nucleus and regulate activation of the transcription factor activator protein-1 (AP-1). In contrast, phosphoinositide 3-kinase (PI3K), which catalyzes the formation of phosphatidylinositol 3,4,5-triphosphate (PIP3) from phosphatidylinositol 4,5-diphosphate [PI(4,5)P2], has been linked with cell survival through the inhibition of the proapoptotic pathway, and with regulation of cell cycle and cell growth. Once activated by receptor tyrosine kinase or G protein–coupled receptors, the generated PIP3 strongly activates PIP3-dependent protein kinase (PDK), leading to activation of Akt.

Figure 1 shows the effects of iAsIII on the alterations in signal transduction pathways and transcription factors in a variety of cells. Arsenic-mediated activation of

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

MAPK signaling through the EGFR/MEK, EGFR/Ras/MEK or Src/EGFR cascade has been identified in BEAS-2B cells, HaCaT cells, mouse epidermal JB6 cells, PC12 cells, Rat1 cells, and UROtsa cells (32–37). This phenomenon was also seen during exposure to iAsV (35, 38, 39) because iAsV incorporated into the cells undergoes reduction by arsenate reductase to iAsIII (40). The MAPKs phosphorylation caused by iAsIII is, at least in part, attributable to reactive oxygen species during arsenic exposure. However, it should be noted that arsenic-mediated activation of transcription factors occurs through different MAPK signaling pathways and thus influences distinct downstream gene expression. For example, in the HaCaT cells, stimulation of p38 MAPK and Akt phosphorylation by iAsIII exposure resulted in eNOS phosphorylation, without EGFR activation (41). Upregulation of HIF-1 caused by iAsIII is dependent on p38 MAPK, but not the ERK1/2, SAPK/JNK, and Akt pathways (42, 43). Growth factor/Ras is involved in iAsIII-induced ERK1/2 activation (32). In UROtsa cells, iAsIII as well as the MMeAsIII and dimethylarsinous acid (DMeAsIII)-activated ERK1/2 pathway were involved in activation of the transcription factor AP-1, which is composed of Jun-Jun homodimer or Jun-Fos heterodimer (44). Alternatively, it was also reported that not only ERK but also protein kinase C (PKC) participate in the activation of AP-1 caused by inorganic arsenic compounds (35). Overall, these observations suggest that arsenic causes AP-1 activation through predominantly MAPK signaling, resulting in apoptosis.

Transcription factor NF-κB is involved in cellular responses to endotoxin, inflammation, and viral infection. In the absence of environmental stress, resting NF-κB is maintained in the cytoplasm through the interaction with inhibitory-κB (IκB) protein. Once activated, IκB kinase (IKK) phosphorylates IκB protein, leading to dissociation, and NF-κB moves into the nucleus. The translocated-NF-κB binds to a κB binding site on the promoter region and stimulates gene expression of cytokines, chemokines, transcriptional factors, and adhesion molecules. TNFα is used as a NF-κB stimulator in various cells. If a reactive thiol (Cys179) of IKK undergoes modification by reactive oxygen species or electrophiles, translocation of constitutive NF-κB into nuclei is suppressed, thereby disrupting the role of NF-κB as an antiapoptotic factor.

In A549, BEAS-2B, and HEK293 cells, arsenite is shown to inhibit TNFα-induced NF-κB activation through action on IKK (45, 46). Arsenite-mediated suppression of NF-κB activation has been attributed to the inhibition of IKK activity through covalent binding to Cys179 in the activation loop of IKK (47). Suppression of constitutive activation of NF-κB during exposure of Hodgkin/Reed-Sternberg (HRS) cells to iAsIII contributes to the induction of apoptosis because constitutive NF-κB activation is essential for cell growth and proliferation (48–50). This finding raises the possibility that arsenite-induced apoptosis through the inhibition of NF-κB in HRS cells might be an effective therapy against Hodgkin lymphoma (50). Inhibition of PI3K/Akt signaling by arsenite accounts for substantial induction of apoptosis as well (51–53). Nevertheless, attention should be paid to the report that iAsIII also acts as an activator of NF-κB through reactive oxygen species (54–56), suggesting an initial response against possibly apoptosis caused by arsenic. Such controversial observations, perhaps reflecting a cell-specificity, make it difficult to understand arsenic-mediated effects on cell signaling and cell death.

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

ACTIVATION OF NRF2

As described above, arsenic-mediated activation (or inactivation) of signal transduction pathways leading to alterations in transcription factors associated with cell growth and apoptosis has been identified. On the one hand, arsenic causes oxidative stress, as determined by 8-OHdG formation (57, 58), lipid peroxide production (59) through reactive oxygen species generation, reduction of glutathione (GSH) content (10, 60), and increased levels of antioxidant proteins such as heme oxygenase-1 (HO-1), A170, and peroxiredoxin 1 (PrxI) (61). On the other hand, arsenic-mediated cytotoxicity is thought to be due to high accumulation of this metalloid in the cells. Thus, it is likely that mammals, including humans, would possess some transcription factor(s) regulating proteins that play a critical role in the cellular defense against oxidative stress and the cellular accumulation of arsenic.

Nuclear factor–erythroid 2–related factor 2 (Nrf2) is a basic-leucine zipper transcription factor that activates the antioxidant responsive element (ARE) and electrophilic responsive element (EpRE), thereby upregulating the expression of a variety of downstream genes (62). Under basal conditions, Nrf2 is bound to Kelch-like ECH-associated protein 1 (Keap1) in an inactive complex (63). It has been reported that Keap1 stimulates Nrf2 degradation though the activation of Cul3-dependent ubiquitination (64). However, once Keap1 is modified by electrophiles or chemicals that generate reactive oxygen species, Nrf2 is readily dissociated from Keap1, leading to its translocation from the cytoplasm to the nucleus (65). Then, the heterodimer of Nrf2 and small Maf protein binds to the ARE on the promoter region and stimulates gene expression of downstream proteins, including antioxidant proteins, Phase II xenobiotic-metabolizing enzymes, and Phase III transporters. A point we would like to emphasize here is that alterations in transcription factors such as activation of AP-1, inhibition of NF-κB, and activation of Nrf2 during arsenic exposure could occur through (*a*) reactive oxygen species production and/or (*b*) an electrophilic metabolite of arsenic (e.g., MMeAsIII) that reacts readily with the reactive thiols of Keap1 (see **Figure 1**).

In early work on arsenic-dependent gene expression, it was reported that multiple basic-leucine zipper transcription factors, including Nrf2, participate in the gene expression of HO-1 (66, 67). With Nrf2 knockout mice, Ishii and his associates found that Nrf2 deletion suppresses induction of the antioxidant proteins, HO-1 and PrxI, caused by arsenite in mouse macrophages (68). Subsequently, we reported that exposure to inorganic arsenic activates Nrf2 in MC-3T3E osteoblast cells and HaCaT cells, resulting in the upregulation of HO-1, PrxI, A170, NAD(P)H-quinone oxidoreductase 1 (NQO1), and γ-glutamylcysteine synthase (γGCS) (61, 69). Experiments with scavenging agents for reactive oxygen species revealed that Nrf2 activation in HaCaT cells caused by arsenite is, at least in part, due to excess reactive oxygen species, especially hydrogen peroxide during arsenic exposure (69). **Figure 2** summarizes (*a*) arsenic accumulation, (*b*) activation of Nrf2, (*c*) upregulation of antioxidant proteins, and (*d*, *e*) luciferase activity through ARE in mouse macrophage RAW 264.7 cells following arsenite exposure (Y. Shinkai & D. Sumi, unpublished data). In RAW 264.7 cells, iAsIII stimulates Nrf2 translocation to the nucleus as shown by nuclear

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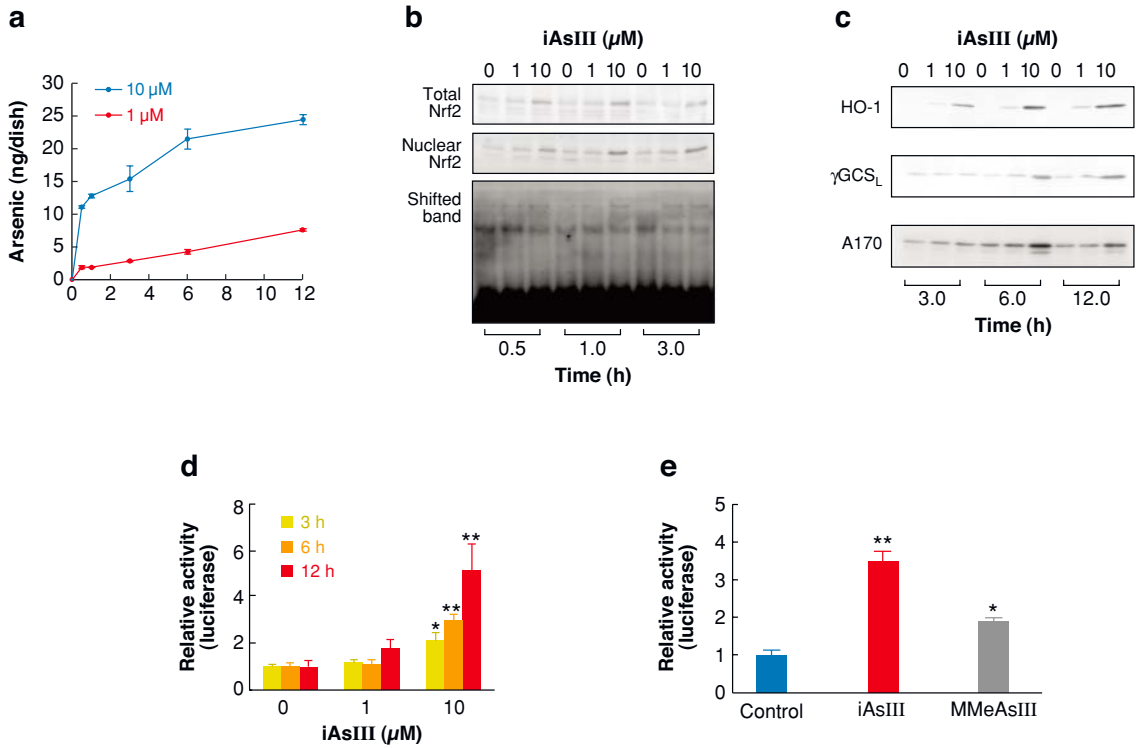

Figure 2

Arsenic activates Nrf2, leading to upregulation of antioxidant protein expression in RAW 264.7 cell. (*a*) Arsenic uptake into the cells by incubation with 1 μM iAsIII (*red points*) or 10 μM iAsIII (*blue points*) for the indicated times. (*b*) Western blot analysis was done on total lysate or nuclear fraction with anti-Nrf2 antibody, and the gel shift mobility shift assay was done with HO-1 ARE probe (67). (*c*) Western blot analysis was done with anti-HO-1 or γGCS_L antibody. (*d*) Luciferase reporter plasmid containing the GSTA1 promoter construct was transiently transfected into RAW 264.7 cell, and the luciferase activities were measured after treatment with 1 or 10 μM iAsIII for 3 (*yellow bars*), 6 (*orange bars*), or 12 h (*red bars*). Luciferase activities were normalized by cotransfecting *Renilla* luciferase control vectors. Each value represents the means ±SD (n = 3). $^*P < 0.05$, $^{**}P < 0.01$ versus control. (*e*) Luciferase activities were measured in the presence of iAsIII (10 μM, *red bar*) or MMeAsIII (2 μM, *gray bar*) for 6 h. Each value represents the means ±SD (n = 3). $^*P < 0.05$, $^{**}P < 0.01$ versus control.

accumulation of Nrf2 protein, followed by binding to the ARE, induction of HO-1, γGCS_L, and A170 protein in a concentration- and time-dependent manner. Slight activation of ARE and negligible changes in Nrf2 and downstream protein levels were seen after exposure to MMeAsIII, suggesting that reactive oxygen species–mediated oxidation of reactive thiols of Keap1 rather than a nucleophilic attack of MMeAsIII to the Keap1 thiols is required for Nrf2 activation in these cells. In contrast, minimal activation of Nrf2, resulting in upregulation of antioxidant proteins, is detected

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

after arsenite exposure at toxic concentrations (>100 μM) (data not shown). This observation implies a breakdown of cellular defenses against this metalloid. Although modification of reactive cysteine in Keap1 is required for Nrf2 activation, some investigators have reported that a different signaling pathway such as PKC is involved in Nrf2 activation (70). Consistent with this notion, arsenate-mediated Nrf2 activation and PrxI induction are dependent on PKC δ (71).

ROLE OF METABOLISM IN TOXICITY

Figure 3 shows the biological fate of inorganic arsenic in the body. In mammalian systems, iAsIII is taken up into cells through aquaporin isozyme 7 or 9 (AQP7/9), a member of aquaglyceroporins (72–74). In the case of iAsV, however, phosphate transporters are thought to act to incorporate arsenate into cells (75). To date, two possible metabolic pathways of inorganic arsenic have been proposed: A classical pathway is reduction and oxidative methylation (76, 77) and a novel pathway is GSH conjugation of iAsIII to form arsenite triglutathione [$As(SG)_3$], and subsequent methylation

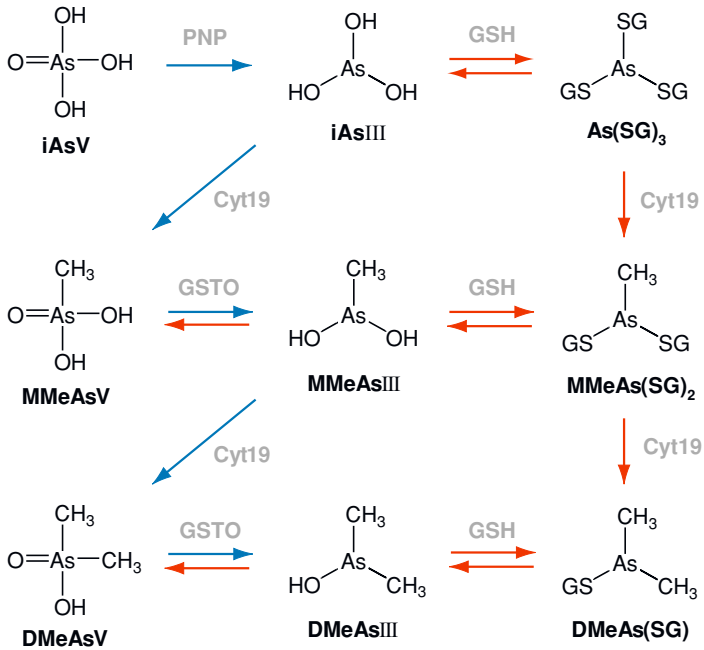

Figure 3

Biotransformation of inorganic arsenic. The blue arrow and red arrow indicate classical and novel pathways of arsenic, respectively. iAsV, inorganic arsenate; iAsIII, inorganic arsenite; MMeAsV, monomethylarsonate; MMeAsIII, monomethylarsonous acid; DMeAsV, dimethylarsinic acid; DMeAsIII, dimethylarsinous acid; $As(SG)_3$, arsenite triglutathione; $MMeAs(SG)_2$, monomethylarsonic diglutathione; DMeAs(SG), dimethylarsinic glutathione; PNP, purine nucleoside phosphorylase; Cyt19, arsenic methyltransferase; GSTO, glutathione *S*-transferase omega; GSH, glutathione.

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

to yield a monomethylarsonic diglutathione [MMeAs(SG)$_2$] followed by dimethylarsinic glutathione [DMeAs(SG)] (78). In the former pathway, arsenate reductases, such as the omega isoform of GSH *S*-transferase (GSTomega) (79–81) and purine nucleoside phosphorylase (PNP) (82, 83), catalyze the reduction of arsenate species, including organic arsenicals to arsenite, and arsenite/MMeAsIII methyltransferase (84–86) and Cyt19 (87) catalyze the methylation of iAsIII and MMeAsIII. Because arsenite species are more toxic than arsenates, variation in the enzyme activity of GSTomega isoform 1, which is identical to monomethylarsonate (MMeAsV) reductase, could influence arsenic toxicity, as suggested by Aposhian and his associates (88). Interestingly, for example, MMeAs(SG)$_2$ produced in the latter pathway (78) undergoes (*a*) spontaneous degradation to form MMeAsIII, followed by MMeAsV via an unidentified oxidation process, or (*b*) further methylation by Cyt19 to produce DMeAs(SG), followed by dimethylarsinous acid (DMeAsIII) to dimethylarsinic acid (DMeAsV), a major metabolite found in urine (89, 90). The enzyme-catalyzed methylation of arsenic requires *S*-adenosylmethionine as the methyl donor and reducing agents such as GSH and cysteine (91). This requirement supports the suggestion by Hayakawa that the iAsIII-thiol adduct is an intermediate for the Cyt19-catalyzed methylation of arsenic (78). Finally, As(SG)$_3$ and MMeAs(SG)$_2$, but not DMeAs(SG), are transported out by multidrug-resistance proteins (MRPs) (92, 93), as shown in **Figure 4**, so these GSH adducts are found in bile of rats treated with arsenic (94, 95). There is little information on the contribution of MRPs to methylated arsenical efflux. As(SG)$_3$ and MMeAs(SG)$_2$ are reported to be unstable in the bile and readily undergo hydrolysis to iAsIII and MMeAsIII, respectively (96), as shown in **Figure 3**. Such a reversible interaction of MMeAsIII with GSH is also suggested (97). Thus, a possible explanation for this discrepancy is that iAsIII and MMeAsIII,

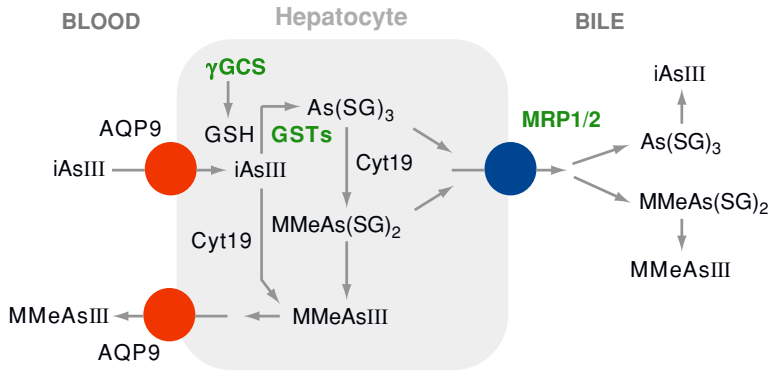

Figure 4

Proposed pathways of transporters for uptake and efflux of arsenites and enzymes responsible for arsenic excretion into extracellular space in hepatocytes. iAsIII, inorganic arsenite; MMeAsIII, monomethylarsonous acid; As(SG)$_3$, arsenite triglutathione; MMeAs(SG)$_2$, monomethylarsonic diglutathione; Cyt19, arsenic methyltransferase; γGCS, γglutamylcysteine synthase; GSTs, glutathione *S*-transferases; GSH, glutathione; AQP9, aquaglyceroporin 9. Proteins (*green*) are regulated by Nrf2.

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

found in the bile as main species after administration of iAsIII to rats (94), may be the result of spontaneous decomposition of these GSH adducts that were transported into the bile by MRPs (**Figure 4**). Although the MRPs responsible for transporting intracellular (e.g., hepatocytes) arsenic out to the blood stream are poorly understood, Rosen and his associates have recently proposed a model in which AQP9 catalyzes a key step in the uptake of iAsIII and efflux of MMeAsIII (98), as shown in **Figure 4**.

The methylation of inorganic arsenicals has long been understood to be a detoxification process of the metalloid (99). However, a key development was the synthesis and crystallization of methylated arsenite, which was postulated to be an intermediate in arsenic metabolism (100). Using the authentic chemicals as standards, methylated metabolites of arsenic as MMeAsIII and DMeAsIII were found as urinary excretion products after exposure of humans to inorganic arsenic (101–105). Surprisingly, MMeAsIII and DMeAsIII are more toxic than inorganic arsenic (106). MMeAsIII is a potent inhibitor of glutathione peroxidase, glutathione reductase (107), pyruvate dehydrogenase (108), and thioredoxin reductase (109). We also found that MMeAsIII, but not MMeAsV or DMeAsV, interacts with endothelial NO synthase (26) and protein tyrosine phosphatase 1B (N. Iwamoto, unpublished data) under conditions that are blocked by dithiothreitol, suggesting that this reactive metabolite of arsenic affects endothelial NO synthase and protein tyrosine phosphatase 1B through covalent attachment to the reactive thiols (see **Figure 1**). Therefore, this methylation has just the opposite consequence for arsenic because other methylated arsenics are not toxic and reactive. Although further study is needed, we speculate that, in spite of their highly reactive characteristics, methylated arsenites generated from iAsIII appear to be obligatory intermediates to the end products of arsenic, such as MMeAsV and DMeAsV. In this context, Wood et al. have recently shown that Cyt19 polymorphism may be involved in the variation of arsenic metabolism, thereby influencing arsenic toxicity in humans (110).

When there is a high concentration of arsenite in the cell, GSH is consumed and γGCS, the rate-limiting enzyme for GSH synthesis, is upregulated. It is probable that there is not only a chemical process (92, 111) but also an enzymatic reaction, catalyzed by GST isozymes, that generates $As(SG)_3$. The GSH adduct is required to remove arsenic from the cell through the MRPs (92). Because blockage of γGCS, GSTs, or MRPs exacerbated iAsIII toxicity in the cells (112, 113), it seems likely that γGCS, GSTs, and MRPs, controlled by Nrf2, play a critical function in arsenic efflux into extracellular space to decrease its toxicity, as shown in **Figure 4**. As expected, Nrf2 deletion increases arsenite-mediated cytotoxicity (Y. Shinkai, unpublished data) (**Figure 5**). In wild-type mice, arsenic upregulates γGCS, GSTA1, and MRP1 as well (**Figure 5**). Increased gene expression of MRP2 that is regulated by Nrf2 during arsenic exposure in vivo is also seen (95, 114). By contrast, pretreatment with sulforaphane, an Nrf2 activator, diminishes cellular accumulation of arsenic, thereby reducing its cellular toxicity in primary mouse hepatocytes (113) (**Figure 5**), which supports the notion that Nrf2 is a critical transcription factor against arsenic toxicity.

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

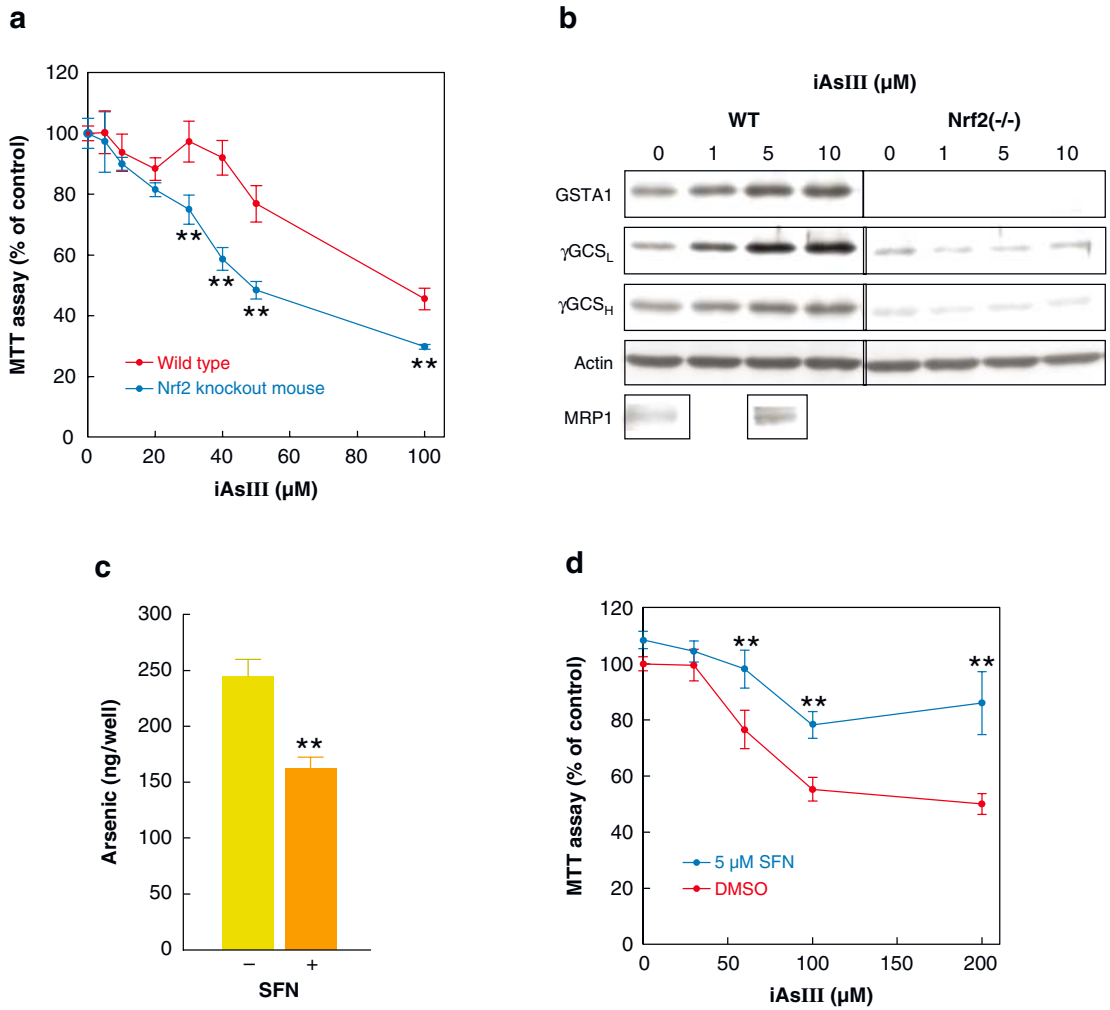

Figure 5

Nrf2 plays a protective role against arsenic-induced cytotoxicity. SFN, Sulforaphane. (*a*) Arsenic-induced cytotoxicity in primary mouse hepatocytes extracted from wild-type (*red points*) or Nrf2 knockout (*blue points*) mouse. Each value represents the means ±SD (n = 4). $^{**}P < 0.01$ versus wild type. (*b*) Western blot analysis was done with antibodies against GSTA1, γGCS_H, γGCS_L, MRP1, and actin. Cells extracted from wild-type or Nrf2 knockout mice [Nrf2(−/−)] were incubated for 24 h in the absence or presence of different concentrations of iAsIII. (*c*) SFN reduced arsenic accumulation in primary mouse hepatocytes. Cells were incubated with either DMSO (0.1%, *yellow bar*) or SFN (5 μM, *orange bar*) for 24 h prior to exposure with 5 μM iAsIII for 24 h. Each value represents the means ±SD (n = 3). $^{**}P < 0.01$ versus DMSO. (*d*) SFN reduced iAsIII-induced cytotoxicity in primary mouse hepatocytes. Cells were incubated with either DMSO (*red points*) or 5 μM SFN (*blue points*) for 24 h prior to the exposure to the indicated iAsIII concentrations. Each value represents the means ±SD (n = 6). $^{**}P < 0.01$ versus DMSO.

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

CONCLUSION

Cellular stress, mediated by an arsenic, leads to an intermediate adaptive response involving various mechanisms, including the nuclear translocation of redox-sensitive transcription factor such as Nrf2, which senses chemical dangers and orchestrates cell defense. In the case of arsenic, Nrf2 genes of immediate significance are the antioxidant proteins, such as HO-1, Prx1, and γGCS; Phase II xenobiotic-metabolizing enzymes; and Phase III transporters, such as GSTA1 and MRP1. It has been noted that nuclear translocation occurs at nontoxic concentrations of arsenic and at timepoints before overt toxicity is observed. However, as arsenic concentration increases, there is a progressive dislocation of nuclear translocation of Nrf2, and gene expression of proteins overwhelms cell defenses. Arsenic exposure can promote apoptosis by disruption of signal transduction, activation of AP-1, inactivation of constitutive NF-κB, and/or suppression of PI3K/Akt signaling. Because upstream proteins that regulate AP-1, NF-κB, and Nrf2 and NO synthase all have reactive thiols (see **Figure 1**), their oxidative modification by reactive oxygen species, generated during arsenic exposure, may be the critical step mediating cellular responses and toxicity.

An intervention study conducted by us in an endemic area of chronic arsenic poisoning in Inner Mongolia suggested that decreased NO levels and peripheral vascular disease in arsenosis patients can be reversed by exposure cessation (115). There is little doubt that arsenic exposure causes adaptive responses against oxidative stress and arsenic cytotoxicity through Nrf2 activation. In areas where substantial accumulation of arsenic in the body occurs through the daily intake of well water containing high concentrations of arsenic, multiple adverse health effects are observed. We suggest that treatment with an Nrf2 activator such as sulforaphane (113) may be an effective strategy for the treatment of chronic arsenic poisoning. In the future, a discovery of a specific inhibitor for AQP9 mediating arsenic uptake into cells would be one of the important topics for improvement of chronic arsenosis.

ACKNOWLEDGMENTS

We wish to thank Yasuhiro Shinkai for preparation of the review article and Prof. Ken Itoh (Center for Advanced Medical Research, Hirosaki University School of Medicine) and Prof. Masayuki Yamamoto (Doctoral Programs in Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba) for kind donation of Nrf2 knockout mice and their earnest encouragement of our study. We would like to thank Dr. William R. Cullen (University of British Columbia) for supplying custom-synthesized MMeAsIII. The work described in this paper was supported in part by the Japan-China Association, the Sumitomo Foundation, and the Grants-in-Aids for Specific Research from the Ministry of Education, Science and Culture of Japan (#13576029, #15406004).

LITERATURE CITED

1. Nordstrom DK. 2002. Public health. Worldwide occurrences of arsenic in ground water. *Science* 296:2143–45

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

2. Smedley PL, Kinniburgh DG. 2002. A review of the source, behavior and distribution of arsenic in natural waters. *Appl. Geochem*. 17:517–68
3. Tseng WP, Chu HM, How SW, Fong JM, Lin CS, Yeh S. 1968. Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. *J. Natl. Cancer Inst*. 40:453–63
4. Lagerkvist BE, Linderholm H, Nordberg GF. 1988. Arsenic and Raynaud's phenomenon. Vasospastic tendency and excretion of arsenic in smelter workers before and after the summer vacation. *Int. Arch. Occup. Environ. Health* 60:361–64
5. Engel RR, Hopenhayn-Rich C, Receveur O, Smith AH. 1994. Vascular effects of chronic arsenic exposure: a review. *Epidemiol. Rev*. 16:184–209
6. Chen CJ, Hsueh YM, Lai MS, Shyu MP, Chen SY, et al. 1995. Increased prevalence of hypertension and long-term arsenic exposure. *Hypertension* 25:53–60
7. Kadono T, Inaoka T, Murayama N, Ushijima K, Nagano M, et al. 2002. Skin manifestations of arsenicosis in two villages in Bangladesh. *Int. J. Dermatol.* 41:841–46
8. Flora SJ. 1999. Arsenic-induced oxidative stress and its reversibility following combined administration of N-acetylcysteine and meso 2,3-dimercaptosuccinic acid in rats. *Clin. Exp. Pharmacol. Physiol*. 26:865–69
9. Wu MM, Chiou HY, Wang TW, Hsueh YM, Wang IH, et al. 2001. Association of blood arsenic levels with increased reactive oxidants and decreased antioxidant capacity in a human population of northeastern Taiwan. *Environ. Health Perspect.* 109:1011–17
10. Pi J, Yamauchi H, Kumagai Y, Sun G, Yoshida T, et al. 2002. Evidence for induction of oxidative stress caused by chronic exposure of Chinese residents to arsenic contained in drinking water. *Environ. Health Perspect.* 110:331–36
11. Pi J, Horiguchi S, Sun Y, Nikaido M, Shimojo N, et al. 2003. A potential mechanism for the impairment of nitric oxide formation caused by prolonged oral exposure to arsenate in rabbits. *Free Radic. Biol. Med.* 35:102–13
12. Pi J, Kumagai Y, Sun G, Yamauchi H, Yoshida T, et al. 2000. Decreased serum concentrations of nitric oxide metabolites among Chinese in an endemic area of chronic arsenic poisoning in inner Mongolia. *Free Radic. Biol. Med.* 28:1137–42
13. Lee MY, Jung BI, Chung SM, Bae ON, Lee JY, et al. 2003. Arsenic-induced dysfunction in relaxation of blood vessels. *Environ. Health Persp.* 111:513–17
14. Lynn S, Gurr JR, Lai HT, Jan KY. 2000. NADH oxidase activation is involved in arsenite-induced oxidative DNA damage in human vascular smooth muscle cells. *Circ. Res*. 86:514–19
15. Smith KR, Klei LR, Barchowsky A. 2001. Arsenite stimulates plasma membrane NADPH oxidase in vascular endothelial cells. *Am. J. Physiol. Lung Cell Mol. Physiol*. 280:L442–49
16. Qian Y, Liu KJ, Chen Y, Flynn DC, Castranova V, Shi X. 2005. Cdc42 regulates arsenic-induced NADPH oxidase activation and cell migration through actin filament reorganization. *J. Biol. Chem*. 280:3875–84

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

17. Heinzel B, John M, Klatt P, Bohme E, Mayer B. 1992. Ca2+/calmodulin-dependent formation of hydrogen peroxide by brain nitric oxide synthase. *Biochem. J.* 281(Pt. 3):627–30
18. Pou S, Pou WS, Bredt DS, Snyder SH, Rosen GM. 1992. Generation of superoxide by purified brain nitric oxide synthase. *J. Biol. Chem.* 267:24173–76
19. Nakamura K, Bindokas VP, Kowlessur D, Elas M, Milstien S, et al. 2001. Tetrahydrobiopterin scavenges superoxide in dopaminergic neurons. *J. Biol. Chem.* 276:34402–7
20. Huie RE, Padmaja S. 1993. The reaction of NO with superoxide. *Free Radic. Res. Commun.* 18:195–99
21. Czapski G, Goldstein S. 1995. The role of the reactions of ·NO with superoxide and oxygen in biological systems: a kinetic approach. *Free Radic. Biol. Med.* 19:785–94
22. Kissner R, Nauser T, Bugnon P, Lye PG, Koppenol WH. 1997. Formation and properties of peroxynitrite as studied by laser flash photolysis, high-pressure stopped-flow technique, and pulse radiolysis. *Chem. Res. Toxicol.* 10:1285–92
23. White CR, Brock TA, Chang LY, Crapo J, Briscoe P, et al. 1994. Superoxide and peroxynitrite in atherosclerosis. *Proc. Natl. Acad. Sci. USA* 91:1044–48
24. Hayashi T, Yamada K, Esaki T, Kano H, Asai Y, et al. 1999. Endothelium-dependent relaxation of rabbit atherosclerotic aorta was not restored by control of hyperlipidemia: the possible role of peroxynitrite (ONOO(-)). *Atherosclerosis* 147:349–63
25. Bunderson M, Brooks DM, Walker DL, Rosenfeld ME, Coffin JD, Beall HD. 2004. Arsenic exposure exacerbates atherosclerotic plaque formation and increases nitrotyrosine and leukotriene biosynthesis. *Toxicol. Appl. Pharmacol.* 201:32–39
26. Sumi D, Taguchi K, Sun Y, Shinkai Y, Kumagai Y. 2005. Monomethylarsonous acid inhibits endothelial nitric oxide synthase activity. *J. Health Sci.* 51:728–30
27. Martindale JL, Holbrook NJ. 2002. Cellular response to oxidative stress: signaling for suicide and survival. *J. Cell Physiol.* 192:1–15
28. Lohse DL, Denu JM, Santoro N, Dixon JE. 1997. Roles of aspartic acid-181 and serine-222 in intermediate formation and hydrolysis of the mammalian protein-tyrosine-phosphatase PTP1. *Biochemistry* 36:4568–75
29. Garrington TP, Johnson GL. 1999. Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr. Opin. Cell Biol.* 11:211–18
30. Tournier C, Hess P, Yang DD, Xu J, Turner TK, et al. 2000. Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. *Science* 288:870–74
31. Sumbayev VV, Yasinska IM. 2005. Regulation of MAP kinase-dependent apoptotic pathway: implication of reactive oxygen and nitrogen species. *Arch. Biochem. Biophys.* 436:406–12
32. Liu Y, Guyton KZ, Gorospe M, Xu Q, Lee JC, Holbrook NJ. 1996. Differential activation of ERK, JNK/SAPK and P38/CSBP/RK map kinase family members during the cellular response to arsenite. *Free Radic. Biol. Med.* 21:771–81

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

33. Chen W, Martindale JL, Holbrook NJ, Liu Y. 1998. Tumor promoter arsenite activates extracellular signal-regulated kinase through a signaling pathway mediated by epidermal growth factor receptor and Shc. *Mol. Cell Biol*. 18:5178–88
34. Samet JM, Graves LM, Quay J, Dailey LA, Devlin RB, et al. 1998. Activation of MAPKs in human bronchial epithelial cells exposed to metals. *Am. J. Physiol.* 275:L551–58
35. Huang C, Bode AM, Chen NY, Ma WY, Li J, et al. 2001. Transactivation of AP-1 in AP-1-luciferase reporter transgenic mice by arsenite and arsenate. *Anticancer Res.* 21:261–67
36. Simeonova PP, Wang S, Hulderman T, Luster MI. 2002. c-Src-dependent activation of the epidermal growth factor receptor and mitogen-activated protein kinase pathway by arsenic. Role in carcinogenesis. *J. Biol. Chem*. 277:2945–50
37. Cooper KL, Myers TA, Rosenberg M, Chavez M, Hudson LG. 2004. Roles of mitogen activated protein kinases and EGF receptor in arsenite-stimulated matrix metalloproteinase-9 production. *Toxic. Appl. Pharmacol*. 200:177–85
38. Huang C, Ma WY, Li J, Dong Z. 1999. Arsenic induces apoptosis through a c-Jun NH2-terminal kinase-dependent, p53-independent pathway. *Cancer Res.* 59:3053–58
39. Porter AC, Fanger GR, Vaillancourt RR. 1999. Signal transduction pathways regulated by arsenate and arsenite. *Oncogene* 18:7794–802
40. Rosen BP. 2002. Biochemistry of arsenic detoxification. *FEBS Lett*. 529:86–92
41. Souza K, Maddock DA, Zhang Q, Chen J, Chiu C, et al. 2001. Arsenite activation of P13K/AKT cell survival pathway is mediated by p38 in cultured human keratinocytes. *Mol. Med.* 7:767–72
42. Duyndam MC, Hulscher TM, Fontijn D, Pinedo HM, Boven E. 2001. Induction of vascular endothelial growth factor expression and hypoxia-inducible factor 1alpha protein by the oxidative stressor arsenite. *J. Biol. Chem*. 276:48066–76
43. Duyndam MC, Hulscher ST, van der Wall E, Pinedo HM, Boven E. 2003. Evidence for a role of p38 kinase in hypoxia-inducible factor 1-independent induction of vascular endothelial growth factor expression by sodium arsenite. *J. Biol. Chem*. 278:6885–95
44. Drobna Z, Jaspers I, Thomas DJ, Styblo M. 2003. Differential activation of AP-1 in human bladder epithelial cells by inorganic and methylated arsenicals. *FASEB J*. 17:67–69
45. Shumilla JA, Wetterhahn KE, Barchowsky A. 1998. Inhibition of NF-kappa B binding to DNA by chromium, cadmium, mercury, zinc, and arsenite in vitro: evidence of a thiol mechanism. *Arch. Biochem. Biophys*. 349:356–62
46. Roussel RR, Barchowsky A. 2000. Arsenic inhibits NF-kappaB-mediated gene transcription by blocking IkappaB kinase activity and IkappaBalpha phosphorylation and degradation. *Arch. Biochem. Biophys*. 377:204–12
47. Kapahi P, Takahashi T, Natoli G, Adams SR, Chen Y, et al. 2000. Inhibition of NF-kappa B activation by arsenite through reaction with a critical cysteine in the activation loop of Ikappa B kinase. *J. Biol. Chem*. 275:36062–66

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

48. Bargou RC, Leng C, Krappmann D, Emmerich F, Mapara MY, et al. 1996. High-level nuclear NF-kappa B and Oct-2 is a common feature of cultured Hodgkin/Reed-Sternberg cells. *Blood* 87:4340–47
49. Bargou RC, Emmerich F, Krappmann D, Bommert K, Mapara MY, et al. 1997. Constitutive nuclear factor-kappaB-RelA activation is required for proliferation and survival of Hodgkin's disease tumor cells. *J. Clin. Invest.* 100:2961–69
50. Mathas S, Lietz A, Janz M, Hinz M, Jundt F, et al. 2003. Inhibition of NF-kappaB essentially contributes to arsenic-induced apoptosis. *Blood* 102:1028–34
51. Hossain K, Akhand AA, Kawamoto Y, Du J, Takeda K, et al. 2003. Caspase activation is accelerated by the inhibition of arsenite-induced, membrane rafts-dependent Akt activation. *Free Radic. Biol. Med.* 34:598–606
52. Ramos AM, Fernandez C, Amran D, Sancho P, de Blas E, Aller P. 2005. Pharmacologic inhibitors of PI3K/Akt potentiate the apoptotic action of the antileukemic drug arsenic trioxide via glutathione depletion and increased peroxide accumulation in myeloid leukemia cells. *Blood* 105:4013–20
53. Tabellini G, Tazzari PL, Bortul R, Evangelisti C, Billi AM, et al. 2005. Phosphoinositide 3-kinase/Akt inhibition increases arsenic trioxide-induced apoptosis of acute promyelocytic and T-cell leukaemias. *Br. J. Haematol.* 130:716–25
54. Wijeweera JB, Gandolfi AJ, Parrish A, Lantz RC. 2001. Sodium arsenite enhances AP-1 and NFkappaB DNA binding and induces stress protein expression in precision-cut rat lung slices. *Toxicol. Sci.* 61:283–94
55. Hu Y, Jin X, Snow ET. 2002. Effect of arsenic on transcription factor AP-1 and NF-kappaB DNA binding activity and related gene expression. *Toxicol. Lett.* 133:33–45
56. Felix K, Manna SK, Wise K, Barr J, Ramesh GT. 2005. Low levels of arsenite activates nuclear factor-kappaB and activator protein-1 in immortalized mesencephalic cells. *J. Biochem. Mol. Toxicol.* 19:67–77
57. Kessel M, Liu SX, Xu A, Santella R, Hei TK. 2002. Arsenic induces oxidative DNA damage in mammalian cells. *Mol. Cell Biochem.* 234–235:301–8
58. Hu CW, Pan CH, Huang YL, Wu MT, Chang LW, et al. 2006. Effects of arsenic exposure among semiconductor workers: a cautionary note on urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine. *Free Radic. Biol. Med.* 40:1273–78
59. Ramos O, Carrizales L, Yanez L, Mejia J, Batres L, et al. 1995. Arsenic increased lipid peroxidation in rat tissues by a mechanism independent of glutathione levels. *Environ. Health Persp.* 103(Suppl. 1):85–88
60. Maiti S, Chatterjee AK. 2001. Effects on levels of glutathione and some related enzymes in tissues after an acute arsenic exposure in rats and their relationship to dietary protein deficiency. *Arch. Toxicol.* 75:531–7
61. Aono J, Yanagawa T, Itoh K, Li B, Yoshida H, et al. 2003. Activation of Nrf2 and accumulation of ubiquitinated A170 by arsenic in osteoblasts. *Biochem. Biophys. Res. Commun.* 305:271–77
62. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, et al. 1997. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem. Biophys. Res. Commun.* 236:313–22

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

63. Itoh K, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, et al. 1999. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev.* 13:76–86
64. Kobayashi A, Kang MI, Okawa H, Ohtsuji M, Zenke Y, et al. 2004. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol. Cell Biol.* 24:7130–39
65. Kobayashi M, Yamamoto M. 2005. Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. *Antiox. Redox Signal*. 7:385–94
66. Yu R, Chen C, Mo YY, Hebbar V, Owuor ED, et al. 2000. Activation of mitogen-activated protein kinase pathways induces antioxidant response element-mediated gene expression via a Nrf2-dependent mechanism. *J. Biol. Chem.* 275:39907–13
67. Gong P, Stewart D, Hu B, Vinson C, Alam J. 2002. Multiple basic-leucine zipper proteins regulate induction of the mouse heme oxygenase-1 gene by arsenite. *Arch. Biochem. Biophys*. 405:265–74
68. Ishii T, Itoh K, Takahashi S, Sato H, Yanagawa T, et al. 2000. Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. *J. Biol. Chem*. 275:16023–29
69. Pi J, Qu W, Reece JM, Kumagai Y, Waalkes MP. 2003. Transcription factor Nrf2 activation by inorganic arsenic in cultured keratinocytes: involvement of hydrogen peroxide. *Exp. Cell Res*. 290:234–45
70. Huang HC, Nguyen T, Pickett CB. 2000. Regulation of the antioxidant response element by protein kinase C-mediated phosphorylation of NF-E2-related factor 2. *Proc. Natl. Acad. Sci. USA* 97:12475–80
71. Li B, Wang X, Rasheed N, Hu Y, Boast S, et al. 2004. Distinct roles of c-Abl and Atm in oxidative stress response are mediated by protein kinase C delta. *Genes Dev.* 18:1824–37
72. Liu Z, Shen J, Carbrey JM, Mukhopadhyay R, Agre P, Rosen BP. 2002. Arsenite transport by mammalian aquaglyceroporins AQP7 and AQP9. *Proc. Natl. Acad. Sci. USA* 99:6053–58
73. Bhattacharjee H, Carbrey J, Rosen BP, Mukhopadhyay R. 2004. Drug uptake and pharmacological modulation of drug sensitivity in leukemia by AQP9. *Biochem. Biophys. Res. Commun*. 322:836–41
74. Liu Z, Carbrey JM, Agre P, Rosen BP. 2004. Arsenic trioxide uptake by human and rat aquaglyceroporins. *Biochem. Biophys. Res. Commun*. 316:1178–85
75. Csanaky I, Gregus Z. 2001. Effect of phosphate transporter and methylation inhibitor drugs on the disposition of arsenate and arsenite in rats. *Toxicol. Sci*. 63:29–36
76. Cullen WR, Reimer KJ. 1989. Arsenic speciation in the environment. *Chem. Rev*. 89:713–64
77. Healy SM, Wildfang E, Zakharyan RA, Aposhian HV. 1999. Diversity of inorganic arsenite biotransformation. *Biol. Trace Elem. Res*. 68:249–66
78. Hayakawa T, Kobayashi Y, Cui X, Hirano S. 2005. A new metabolic pathway of arsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19. *Arch. Toxicol*. 79:183–91

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

79. Zakharyan RA, Aposhian HV. 1999. Enzymatic reduction of arsenic compounds in mammalian systems: the rate-limiting enzyme of rabbit liver arsenic biotransformation is MMA(V) reductase. *Chem. Res. Toxicol.* 12:1278–83
80. Zakharyan RA, Sampayo-Reyes A, Healy SM, Tsaprailis G, Board PG, et al. 2001. Human monomethylarsonic acid (MMA(V)) reductase is a member of the glutathione-S-transferase superfamily. *Chem. Res. Toxicol.* 14:1051–57
81. Zakharyan RA, Tsaprailis G, Chowdhury UK, Hernandez A, Aposhian HV. 2005. Interactions of sodium selenite, glutathione, arsenic species, and omega class human glutathione transferase. *Chem. Res. Toxicol.* 18:1287–95
82. Radabaugh TR, Sampayo-Reyes A, Zakharyan RA, Aposhian HV. 2002. Arsenate reductase II. Purine nucleoside phosphorylase in the presence of dihydrolipoic acid is a route for reduction of arsenate to arsenite in mammalian systems. *Chem. Res. Toxicol.* 15:692–98
83. Gregus Z, Nemeti B. 2002. Purine nucleoside phosphorylase as a cytosolic arsenate reductase. *Toxicol. Sci.* 70:13–19
84. Zakharyan R, Wu Y, Bogdan GM, Aposhian HV. 1995. Enzymatic methylation of arsenic compounds: assay, partial purification, and properties of arsenite methyltransferase and monomethylarsonic acid methyltransferase of rabbit liver. *Chem. Res. Toxicol.* 8:1029–38
85. Wildfang E, Zakharyan RA, Aposhian HV. 1998. Enzymatic methylation of arsenic compounds. VI. Characterization of hamster liver arsenite and methylarsonic acid methyltransferase activities in vitro. *Toxicol. Appl. Pharmacol.* 152:366–75
86. Zakharyan RA, Ayala-Fierro F, Cullen WR, Carter DM, Aposhian HV. 1999. Enzymatic methylation of arsenic compounds. VII. Monomethylarsonous acid (MMAIII) is the substrate for MMA methyltransferase of rabbit liver and human hepatocytes. *Toxicol. Appl. Pharmacol.* 158:9–15
87. Lin S, Shi Q, Nix FB, Styblo M, Beck MA, et al. 2002. A novel S-adenosyl-L-methionine:arsenic(III) methyltransferase from rat liver cytosol. *J. Biol. Chem.* 277:10795–803
88. Marnell LL, Garcia-Vargas GG, Chowdhury UK, Zakharyan RA, Walsh B, et al. 2003. Polymorphisms in the human monomethylarsonic acid (MMA V) reductase/hGSTO1 gene and changes in urinary arsenic profiles. *Chem. Res. Toxicol.* 16:1507–13
89. Mandal BK, Ogra Y, Anzai K, Suzuki KT. 2004. Speciation of arsenic in biological samples. *Toxicol. Appl. Pharmacol.* 198:307–18
90. Yamauchi H, Yamamura Y. 1985. Metabolism and excretion of orally administrated arsenic trioxide in the hamster. *Toxicology* 34:113–21
91. Waters SB, Devesa V, Del Razo LM, Styblo M, Thomas DJ. 2004. Endogenous reductants support the catalytic function of recombinant rat cyt19, an arsenic methyltransferase. *Chem. Res. Toxicol.* 17:404–9
92. Kala SV, Neely MW, Kala G, Prater CI, Atwood DW, et al. 2000. The MRP2/cMOAT transporter and arsenic-glutathione complex formation are required for biliary excretion of arsenic. *J. Biol. Chem.* 275:33404–8

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

93. Leslie EM, Haimeur A, Waalkes MP. 2004. Arsenic transport by the human multidrug resistance protein 1 (MRP1/ABCC1). Evidence that a tri-glutathione conjugate is required. *J. Biol. Chem*. 279:32700–8
94. Gregus Z, Gyurasics A, Csanaky I. 2000. Biliary and urinary excretion of inorganic arsenic: monomethylarsonous acid as a major biliary metabolite in rats. *Toxicol. Sci*. 56:18–25
95. Cui X, Kobayashi Y, Hayakawa T, Hirano S. 2004. Arsenic speciation in bile and urine following oral and intravenous exposure to inorganic and organic arsenics in rats. *Toxicol. Sci*. 82:478–87
96. Kobayashi Y, Cui X, Hirano S. 2005. Stability of arsenic metabolites, arsenic triglutathione [As(GS)3] and methylarsenic diglutathione [CH3As(GS)2], in rat bile. *Toxicology* 211:115–23
97. Kojima C, Sakurai T, Waalkes MP, Himeno S. 2005. Cytolethality of glutathione conjugates with monomethylarsenic or dimethylarsenic compounds. *Biol. Pharm. Bull*. 28:1827–32
98. Liu Z, Styblo M, Rosen BP. 2006. Methylarsonous acid transport by aquaglyceroporins. *Environ. Health Persp*. 114:527–31
99. Aposhian HV. 1997. Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. *Annu. Rev. Pharmacol. Toxicol*. 37:397–419
100. Cullen WR, McBride BC, Manji H, Pickett AW, Reglinski J. 1989. The metabolism of methylarsine oxide and sulfide. *Appl. Organomet. Chem*. 1:71–78
101. Aposhian HV, Gurzau ES, Le XC, Gurzau A, Healy SM, et al. 2000. Occurrence of monomethylarsonous acid in urine of humans exposed to inorganic arsenic. *Chem. Res. Toxicol.* 13:693–97
102. Le XC, Lu X, Ma M, Cullen WR, Aposhian HV, Zheng B. 2000. Speciation of key arsenic metabolic intermediates in human urine. *Anal. Chem*. 72:5172–77
103. Le XC, Ma M, Cullen WR, Aposhian HV, Lu X, Zheng B. 2000. Determination of monomethylarsonous acid, a key arsenic methylation intermediate, in human urine. *Environ. Health Perspect*. 108:1015–18
104. Mandal BK, Ogra Y, Suzuki KT. 2001. Identification of dimethylarsinous and monomethylarsonous acids in human urine of the arsenic-affected areas in West Bengal, India. *Chem. Res. Toxicol*. 14:371–78
105. Valenzuela OL, Borja-Aburto VH, Garcia-Vargas GG, Cruz-Gonzalez MB, Garcia-Montalvo EA, et al. 2005. Urinary trivalent methylated arsenic species in a population chronically exposed to inorganic arsenic. *Environ. Health Persp.* 113:250–54
106. Styblo M, Drobna Z, Jaspers I, Lin S, Thomas DJ. 2002. The role of biomethylation in toxicity and carcinogenicity of arsenic: a research update. *Environ. Health Persp.* 110(Suppl. 5):767–71
107. Chouchane S, Snow ET. 2001. In vitro effect of arsenical compounds on glutathione-related enzymes. *Chem. Res. Toxicol*. 14:517–22
108. Petrick JS, Jagadish B, Mash EA, Aposhian HV. 2001. Monomethylarsonous acid (MMA(III)) and arsenite: LD(50) in hamsters and in vitro inhibition of pyruvate dehydrogenase. *Chem. Res. Toxicol*. 14:651–56

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

109. Lin S, Del Razo LM, Styblo M, Wang C, Cullen WR, Thomas DJ. 2001. Arsenicals inhibit thioredoxin reductase in cultured rat hepatocytes. *Chem. Res. Toxicol.* 14:305–11
110. Wood TC, Salavagionne OE, Mukherjee B, Wang L, Klumpp AF, et al. 2006. Human arsenic methyltransferase (AS3MT) pharmacogenetics. *J. Biol. Chem.* 281:7364–73
111. Rey NA, Howarth OW, Pereira-Maia EC. 2004. Equilibrium characterization of the As(III)-cysteine and the As(III)-glutathione systems in aqueous solution. *J. Inorg. Biochem.* 98:1151–59
112. Liu J, Chen H, Miller DS, Saavedra JE, Keefer LK, et al. 2001. Overexpression of glutathione S-transferase II and multidrug resistance transport proteins is associated with acquired tolerance to inorganic arsenic. *Mol. Pharmacol.* 60:302–9
113. Shinkai Y, Sumi D, Fukami I, Ishii T, Kumagai Y. 2006. Sulforaphane, an activator of Nrf2, suppresses cellular accumulation of arsenic and its cytotoxicity in primary mouse hepatocytes. *FEBS Lett.* 580:1771–74
114. Vollrath V, Wielandt AM, Iruretagoyena M, Chianale J. 2006. Role of Nrf2 in the regulation of the Mrp2 (ABCC2) gene. *Biochem. J.* 395:599–609
115. Pi J, Yamauchi H, Sun G, Yoshida T, Aikawa H, et al. 2005. Vascular dysfunction in patients with chronic arsenosis can be reversed by reduction of arsenic exposure. *Environ. Health Perspect.* 113:339–41

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

Annual Review of Pharmacology and Toxicology

Volume 47, 2007

Contents

Annu. Rev. Pharmacol. Toxicol. 2007.47:243-262.

Indexes

Errata

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