The Role of Biomethylation in Toxicity and Carcinogenicity of Arsenic: A Research Update

Miroslav Stýblo,[1,2,3] Zuzana Drobná,[1] Ilona Jaspers,[1,3] Shan Lin,[4] and David J. Thomas[5]

[1]Department of Pediatrics, [2]Department of Nutrition, [3]Center for Environmental Medicine and Lung Biology, and [4]Curriculum in Toxicology, University of North Carolina, Chapel Hill, North Carolina, USA; [5]Pharmacokinetics Branch, Experimental Toxicology

Recent research of the metabolism and biological effects of arsenic has profoundly changed our understanding of the role of metabolism in modulation of toxicity and carcinogenicity of this metalloid. Historically, the enzymatic conversion of inorganic arsenic to mono- and dimethylated species has been considered a major mechanism for detoxification of inorganic arsenic. However, compelling experimental evidence obtained from several laboratories suggests that biomethylation, particularly the production of methylated metabolites that contain trivalent arsenic, is a process that activates arsenic as a toxin and a carcinogen. This article summarizes this evidence and provides new data on *a*) the toxicity of methylated trivalent arsenicals in mammalian cells, *b*) the effects of methylated trivalent arsenicals on gene transcription, and *c*) the mechanisms involved in arsenic methylation in animal and human tissues. *Key words:* AP-1, arsenic, cancer, inhibition, methylated arsenic, methylation, methyltransferase, toxicity, transcription control. *Environ Health Perspect* 110(suppl 5):767–771 (2002).
http://ehpnet1.niehs.nih.gov/docs/2002/suppl-5/767-771styblo/abstract.html

The metabolism of inorganic arsenic (iAs) in humans involves two types of chemical reactions, the reduction of pentavalent arsenicals to trivalency and the oxidative methylation of trivalent arsenicals to yield methylated pentavalent metabolites (*1*) (Figure 1). Glutathione (GSH) has been shown to reduce pentavalent arsenicals (arsenate iAs^{V}), methylarsonic acid (MAs^{V}), and dimethylarsinic acid ($DMAs^{V}$) in aqueous solutions (*2,3*). As^{V} reductases may enzymatically reduce these arsenicals in mammalian tissues (*4,5*). Methylation of trivalent arsenicals [arsenite (iAs^{III}) and methylarsonous acid (MAs^{III})] is catalyzed by As^{III}-methyltransferases that use *S*-adenosylmethionine (AdoMet) as the methyl group donor (*6,7*). Because MAs^{V} and $DMAs^{V}$ are not toxic in acute lethality assays, methylation of iAs has long been considered a detoxification mechanism. However, methylated arsenicals that are chemically consistent with trivalent methylated metabolites, MAs^{III} and dimethylarsinous acid ($DMAs^{III}$), have been shown to be more potent enzyme inhibitors and cytotoxins than either iAs^{V} or iAs^{III} (*8*). Diiodomethylarsine ($MAs^{III}I_2$) and methylarsine oxide ($MAs^{III}O$) are potent inhibitors of glutathione disulfide (GSSG) reductase (*9*), pyruvate dehydrogenase (*10*), and especially thioredoxin reductase (*11*). $MAs^{III}O$ and $MAs^{III}I_2$ are also far more toxic than iAs^{III} for various types of mammalian cells (*12–14*). $DMAs^{III}$ derivatives [iododimethylarsine ($DMAs^{III}I$) and dimethylarsinous-glutathione ($DMAs^{III}GS$)] are at least as cytotoxic as iAs^{III} for most cell types examined. Notably, exposures to low concentrations of either $MAs^{III}O$ or $DMAs^{III}I$ induce cell proliferation and production of growth-promoting cytokines in normal human keratinocytes (NHEK) (*15*). Unlike iAs^{III} and iAs^{V}, $MAs^{III}O$ and $DMAs^{III}I$ react directly with DNA, nicking naked DNA *in vitro* and damaging nuclear DNA in intact human leukocytes (*16*). Evidence for the formation of methylated trivalent arsenicals in the course of the metabolism of iAs in humans has been obtained using optimized analytical techniques (*17,18*). MAs^{III} and $DMAs^{III}$ have been detected in urine of individuals chronically exposed to iAs in drinking water (*5,18–20*) and in cultured human hepatic cells exposed to various concentrations of iAs^{III} (*18*). Studies are currently under way in several laboratories to elucidate the role of methylated trivalent metabolites in the systemic toxicity and carcinogenicity of iAs. This report summarizes some recent work linking the metabolism of arsenic to its biological effects.

Toxicity of Methylated Trivalent Arsenicals in Mammalian Cells

Cytotoxic effects of trivalent and pentavalent arsenicals have previously been examined in several cell types, including primary human hepatocytes, primary human bronchial epithelial cells (HBEC), NHEK), SV-40–immortalized human bladder epithelial (UROtsa) cells, HeLa cells (*12,13*), and Chang liver cells (*14*). Pentavalent arsenicals were significantly less cytotoxic than their trivalent counterparts (*12–14*). Among trivalent arsenicals, $MAs^{III}O$ and $MAs^{III}I_2$ were the most cytotoxic species, followed by $DMAs^{III}I$, $DMAs^{III}GS$, and iAs^{III}. We have recently examined cytotoxicity of arsenicals in several other mammalian cell types, including human hepatocellular carcinoma (HepG2) cells, human bladder transient carcinoma (T24) cells, human acute promyelocytic leukemia (NB4) cells, human monoblastoid (U937) cells, human osteosarcoma (HOS) cells, human neuroblastoma (SK-N-SH) cells, mouse 3T3 adipocytes, primary guinea pig hepatocytes, and Chinese hamster lung (V79-4) cells (Table 1). Regardless of the cell type, trivalent monomethylated arsenicals, $MAs^{III}O$ and $MAs^{III}I_2$, were the most potent cytotoxins, with LC_{50} values ranging from 0.4 to 5.5 μM. $DMAs^{III}$ derivatives were as cytotoxic as MAs^{III} species and more cytotoxic than iAs^{III} in most cell types.

The thiazolyl blue (MTT) assay that monitors the activity of mitochondrial dehydrogenases in viable cells has been used to examine cytotoxicity of arsenicals in all these cell types. The neutral red assay that measures the uptake of the die by viable cells has also been used in some experiments. Because the cell viability values determined by the neutral red assay were lower that those obtained by the MTT

This article is part of the monograph *Molecular Mechanisms of Metal Toxicity and Carcinogenicity.*

Address correspondence to M. Stýblo, Dept. of Pediatrics, CB# 7220, Burnett-Womack Clinical Sciences Bldg., University of North Carolina, Chapel Hill, NC 27599-7220 USA. Telephone: (919) 966-5721. Fax: (919) 966-0135. E-mail: styblo@med.unc.edu

Research in the laboratory of M.S. has been supported by Drinking Water STAR grant R826136-01-0 from the U.S. Environmental Protection Agency, National Institutes of Health (NIH) grant ES09941, and NIH Clinical Nutrition Research Center grant DK 56350. We thank W. Cullen for synthesis of methylated trivalent arsenicals and N. Unimye, S. Waxman, T. Rossman, Y. Patel, J. Blatt, M. Mass, and A. Klingerman for providing cell lines used for this research. The manuscript was reviewed in accordance with the policy of the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Received 13 February 2002; accepted 31 May 2002.

assay (*12,15*), it is possible that the latter assay underestimates cytotoxic effects of arsenicals in cultured cells. Figure 2 shows an example of the concentration-dependent effects of trivalent arsenicals on cell viability in human leukemia NB4 and U937 cell lines. Increased cell viability values found after 24-hr exposures to low concentrations of arsenicals were associated with increased cell proliferation rates. The induction of cell proliferation by low concentrations of trivalent arsenicals has previously been reported in several cell types [e.g., NHEK (*15*)]. Notably, among cell types examined, NB4 cells were most sensitive to cytotoxic effects of trivalent arsenicals.

As shown in Table 1, there was no apparent correlation between the capacity of cells to methylate iAs and their sensitivity to the cytotoxic effects of trivalent arsenicals, indicating that the capacity to methylate has little to do with the resistance of cells to acute toxicity of As^{III}. In some cases, iAs^{III} was more toxic in cells with a high methylation capacity (e.g., rat hepatocytes) than in cells that do not methylate this arsenical (e.g., guinea pig hepatocytes). Consequently, mechanisms other than methylation (e.g., transport of arsenicals across the cell membrane or protein binding) may play a critical role in the detoxification of trivalent arsenicals under acute exposure conditions. These results suggest that production and accumulation of MAs^{III} and/or $DMAs^{III}$, the most cytotoxic species among biologically relevant arsenicals, may be directly linked to adverse effects associated with *in vivo* exposures to iAs. We have previously shown that HepG2 cells exposed to iAs^{III} produced both MAs^{III} and $DMAs^{III}$. In addition, both MAs^{III} and $DMAs^{III}$ synthesized in HepG2 cells were released into culture medium (*18*). Hence, MAs^{III} and $DMAs^{III}$ may be translocated from methylating cells to tissues and cells that cannot methylate iAs. Notably, production of MAs^{III} and $DMAs^{III}$ by HepG2 cells increased with increasing concentrations of iAs^{III} in the culture. Similarly, epidemiologic studies have shown that urinary levels of MAs^{III} and $DMAs^{III}$ in individuals exposed to iAs in drinking water are positively correlated with exposure levels (*5,18*). These results suggest that individuals exposed to higher levels of iAs may be at greater risk associated with the production of these toxic methylated metabolites.

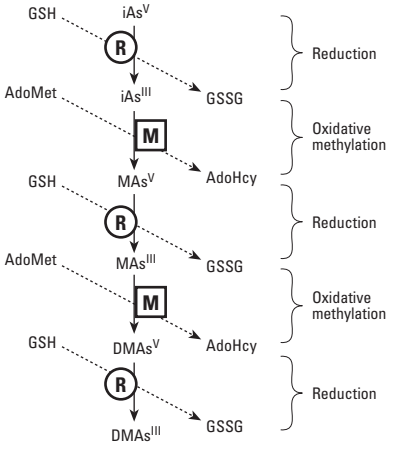

Figure 1. Scheme of the metabolic conversions of iAs in humans. AdoHcy, *S*-adenosylhomocysteine; R, As^{V} reductase; M, As^{III} methyltransferase.

Effects of Methylated Trivalent Arsenicals on Gene Transcription

Various hypotheses have been proposed to explain the carcinogenicity of iAs (*28*). Nevertheless, molecular mechanisms by which this arsenical induces cancer are still poorly understood. Results of previous studies indicated that iAs does not act through classic genotoxic and mutagenic mechanisms, but rather may be a tumor promoter that modifies signal transduction pathways involved in cell growth and proliferation (*29*). iAs^{III} has been shown to modulate expression and/or DNA-binding activities of several key transcription factors, including nuclear factor kappa B (*30*), tumor suppressor 53 (p53) (*31*), and activating protein-1 (AP-1) (*32–34*). Mechanisms of AP-1 activation by iAs^{III} include stimulation of the mitogen-activated protein kinase (MAPK) cascade with a consequent increase in the expression and/or phosphorylation of the two major AP-1 constituents, c-Jun and c-Fos (*29*). The modulation of AP-1–dependent gene transcription by iAs^{III} may contribute to the induction of cell proliferation in cultured cells exposed to this arsenical. However, there are no data on the effects of methylated trivalent arsenicals on AP-1 composition and DNA-binding activity.

Recently, we have examined c-Jun and c-Fos expression and AP-1 DNA-binding

Table 1. Toxicity of trivalent arsenicals in mammalian cells.

Cell type	Estimated LC_{50} values[a] (μM) iAs^{III}	$MAs^{III}O$	$MAs^{III}I_2$	$DMAs^{III}GS$	$DMAs^{III}I$	Methylation rate[b] (pmol As/10^6cells/hr)	References
Primary rat hepatocytes	10–>20	2.8	1.8	14.5	2.7	19	(*12,13*)
Primary human hepatocytes	20	5.5		>20		3.3	(*13*)
NHEK	10–>20	2.6		8.5		0.12	(*12,13*)
HBEC	3.2	2.7		6.8		0.05	(*13*)
UROtsa	17.8	2.0	0.8	14.2	>20	ND[c]	(*12,13*)
HeLa	>20	2.8		>20		0.25	(*12*)
HepG2	>50	4.0			8.0	0.5	(*21*)
T24	>10	2.5			>10	ND	(*21*)
NB4	1.0	0.4			0.4	ND	(*22*)
U937	5.0	1.0			1.0	ND	(*22*)
HOS	25	1.6			7.0	0.25	(*23*)
SK-N-SH	40	2.7			2.0	ND	(*24*)
3T3adipocytes	100	5.0			6.0	0.3	(*25*)
Primary guinea pig hepatocytes	50	3.2			5.0	ND	(*26*)
V79	4.5	0.5			0.4	ND	(*27*)

Abbreviations: ND, not determined. [a]LC_{50} is defined as the concentration of an arsenical that resulted in a 50% decrease in cell viability over a 24-hr incubation period. The MTT assay was used to examine cell viability in all cell types. [b]Methylation rates were determined in cultures exposed to 0.05 or 0.1 μM iAs^{III} for 24 or 48 hr. [c]Methylation activity not detected.

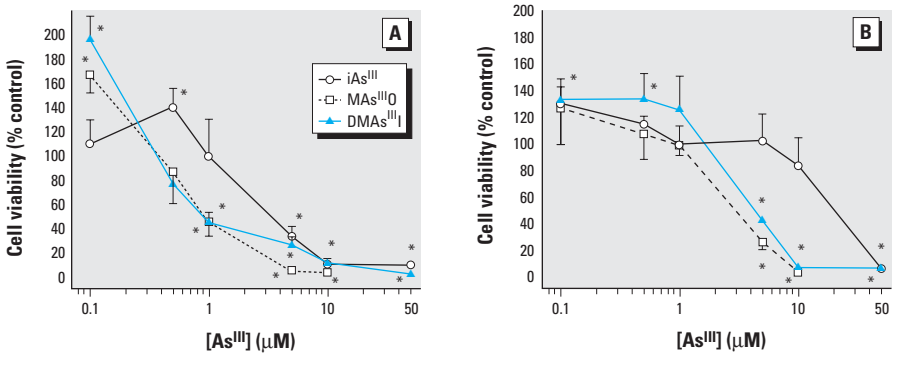

Figure 2. Effects of trivalent arsenicals on cell viability in (*A*) NB4 and (*B*) U937 cultures. Cell viability was determined by the MTT assay after 24-hr exposures to iAs^{III} (circles), $MAs^{III}O$ (squares), or $DMAs^{III}I$ (triangles). Each symbol and error bar represents mean and SD for $n = 4$. Asterisk (*) indicates cell viability in treated cultures is significantly different ($p < 0.05$) from that in untreated cultures as determined by analysis of variance with the Dunnett multiple comparison posttest.

activity in several human cell lines, including UROtsa, T24, HepG2, and primary human hepatocytes exposed to trivalent or pentavalent inorganic or methylated arsenicals. Short-time exposures to trivalent, but not to pentavalent, arsenicals increased AP-1 DNA-binding activity in all these cell types. Most profound effects were found in UROtsa and T24 cells. In these cell lines, exposures to $MAs^{III}O$ or $DMAs^{III}I$ significantly increased the levels of nuclear phospho–c-Jun (p-c-Jun) but had no effects on either c-Jun or c-Fos levels (*35*). Importantly, $MAs^{III}O$ and $DMAs^{III}I$ were considerably more potent inducers of c-Jun phosphorylation and AP-1 activation than was iAs^{III}. Neither iAs^{V} nor methylated pentavalent arsenicals, MAs^{V} or $DMAs^{V}$, modified c-Jun phosphorylation. Figure 3 shows nuclear levels of p-c-Jun in UROtsa cells exposed for 1 hr to iAs^{III}, $MAs^{III}O$, or $DMAs^{III}I$ (0.5, 1, or 5 μM). $MAs^{III}O$ was the most potent inducer of p-c-Jun, followed by $DMAs^{III}I$. In contrast, exposures to iAs^{III} suppressed p-c-Jun levels in this cell line. The AP-1 DNA-binding activity was induced in UROtsa cells exposed to as little as 0.1 μM $MAs^{III}O$ (*35*), a concentration that is well below the LC_{50} value for these cells (Table 1).

The potencies of $MAs^{III}O$ and $DMAs^{III}I$ to stimulate the AP-1–dependent gene transcription have further been demonstrated using UROtsa and T24 cells transiently transfected with an AP-1–dependent promoter-reporter construct (*35*). Somewhat different AP-1 activation patterns were observed in primary human hepatocytes (Figure 4). Among trivalent arsenicals examined, $MAs^{III}O$ was the most potent inducer of c-Jun phosphorylation in these cells. However, only a weak induction of p-c-Jun was observed in human hepatocytes exposed to iAs^{III} or $DMAs^{III}I$. A significant induction of the AP-1 DNA-binding activity was detected by the electrophoretic mobility shift assay (EMSA) only in cells exposed to 5 μM $MAs^{III}O$ (Figure 5). Under these exposure conditions, p-c-Jun, but not c-Fos, was the major constituent of the AP-1 DNA-binding complex. Based on these results, the AP-1 DNA-binding activity appears to be less sensitive to induction by trivalent arsenicals in primary human hepatocytes than in either UROtsa or T24 cell lines that are derived from human urinary bladder. Accordingly, trivalent arsenicals, particularly MAs^{III}, are likely to induce the AP-1–dependent gene transcription in human bladder to a greater extent than in the liver. Notably, both hepatic and urinary bladder cancers have been associated with chronic exposures to iAs in drinking water. However, the incidence of bladder cancer exceeds that of hepatic cancer (*36–38*). Thus, trivalent methylated arsenicals that are chemically consistent with trivalent methylated metabolites of iAs are more potent than iAs, inducing the DNA-binding activity of AP-1, a key transcription factor that is involved in regulation of cell proliferation and death (*29*).

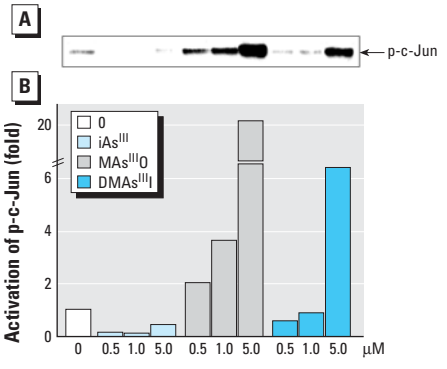

Figure 3. Immunoblot analysis of p-c-Jun in nuclear protein extracts from UROtsa cells exposed to iAs^{III}, $MAs^{III}O$, or $DMAs^{III}I$ for 1 hr and from control (untreated) cells. Nuclear proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and electroblotted on polyvinyl difluoride membranes. Membranes were blocked with 5% nonfat milk, washed, and probed with a mouse monoclonal antibody specific for p-c-Jun (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Blots were visualized by an enhanced chemiluminescence technology and quantified using a digital imaging system. Immunoblot images (*A*) and results of the quantitative analysis of these images (*B*) are shown.

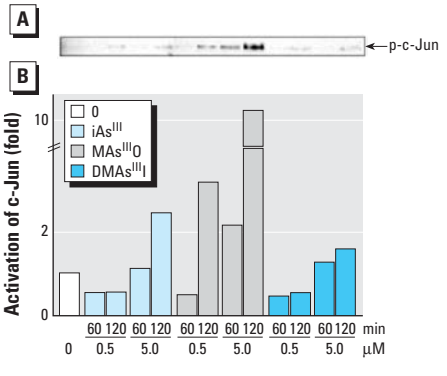

Figure 4. Immunoblot analysis of p-c-Jun in nuclear protein extracts from primary human hepatocytes exposed to iAs^{III}, $MAs^{III}O$, or $DMAs^{III}I$ for 1 or 2 hr and from control (untreated) cells. Immunoblot images (*A*) and results of the quantitative analysis of these images (*B*) are shown. For description of method, see Figure 3.

Mechanism of iAs Methylation

The enzymatic reactions involved in the reduction and methylation of arsenicals have

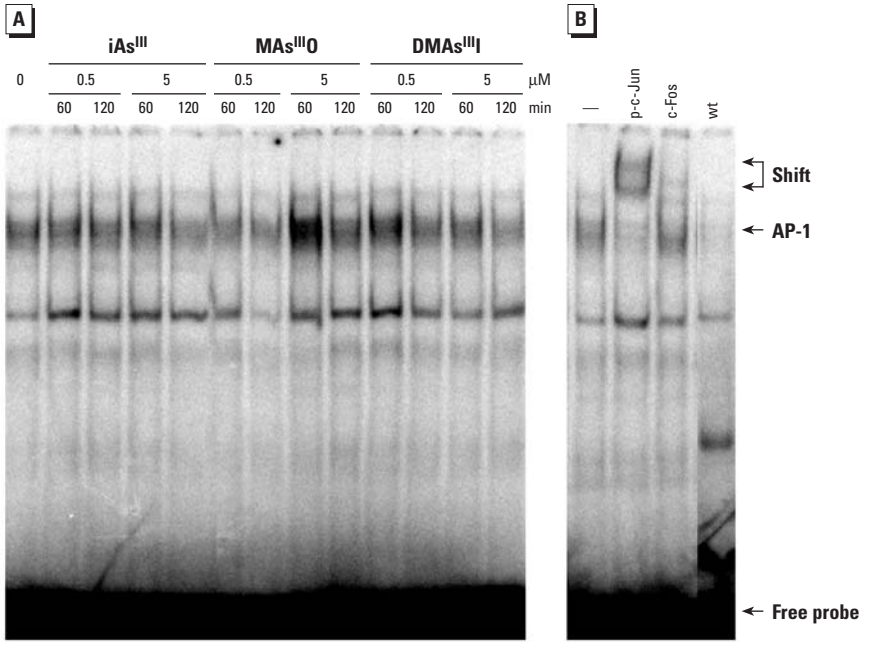

Figure 5. AP-1 DNA-binding activity in primary human hepatocytes treated with trivalent arsenicals and in control (untreated) cells. (*A*) EMSA of nuclear protein extracts from cells treated for 1 or 2 hr with iAs^{III}, $MAs^{III}O$, or $DMAs^{III}I$ and from control cells. The DNA–protein binding assay was performed in a reaction buffer (*28*) containing a radiolabeled AP-1–binding probe (5′-TGAGTCAG-3′; Promega, Madison, WI, USA), nuclear proteins, and poly(dI-dC) · poly(dI-dC) (Boehringer Ingelheim, Ridgefield, CT, USA). The DNA-binding complexes were separated by PAGE, and the distribution of radioactivity was analyzed in dried gels by phosphoimaging. (*B*) EMSA-supershift analysis of a nuclear protein extract from cells treated with 5 μM $MAs^{III}I$ for 1 hr. The reaction and analysis were performed as described for *A*. To identify the AP-1 dimer constituents, antibodies specific for p-c-Jun (lane 2) or for c-Fos (lane 3) (both from Santa Cruz Biotechnology) were added into the DNA–protein binding mixture. The specificity of the assay was established using a 50-fold excess of a wild-type (wt, lane 4) AP-1 probe.

been studied in several laboratories using fractionated tissues, intact cells, and purified enzymes (*8*). Distinct As^V reductases have been shown to catalyze reduction of iAs^V to iAs^{III} and of MAs^V to MAs^{III} (*4,39*). Both these enzymes require thiols (e.g., GSH) for reducing activity. The MAs^V reductase (K_m = 2.6 mM) has recently been identified as GSH-*S*-transferase omega (*40*). Methyltransferases that catalyze methylation of iAs^{III} and MAs^{III} have also been identified. A rabbit liver enzyme that converts iAs^{III} to MAs and $MAs^{III}O$ to DMAs has been purified and partially characterized (*6*). This cytosolic protein has a molecular weight of about 60 kDa and requires both AdoMet and a thiol for activity. Consistent with the metabolic scheme in Figure 1, the purified enzyme has a greater affinity for MAs^{III} than for MAs^V.

A novel As^{III} methyltransferase (M_r = 41 kDa) has recently been purified by Lin and co-workers (*7*) from rat liver. This enzyme methylates iAs^{III} in a two-step reaction, in which MAs is an intermediate and DMAs is the final product. The two-step kinetics of this reaction is consistent with kinetic patterns of iAs^{III} methylation reported in *in vitro* studies using tissue extracts (*41,42*). AdoMet is the essential methyl group donor for both methylation steps (Table 2). $MAs^{III}O$ is also a substrate for this enzyme in a methylation reaction yielding DMAs. A kinetic analysis of this reaction showed a low K_m of 250 nM $MAs^{III}O$. Thus, this enzyme can effectively methylate at very low concentrations of MAs^{III} in tissues. However, high concentrations of $MAs^{III}O$ (≥5 μM) inhibit DMAs synthesis. The rat As^{III} methyltransferase requires a dithiol for its activity. Dithiothreitol (DTT) has been used as an enzyme co-factor in *in vitro* assays with purified rat As^{III} methyltransferase. Protein and cDNA sequences for the rat As^{III} methyltransferase have been obtained. Sequence analyses have revealed a high degree of homology with a putative human methyltransferase CYT19, indicating that CYT19 is the human As^{III} methyltransferase. Using reverse-transcription polymerase chain reaction, mRNA for As^{III} methyltransferase has been detected in rat tissues (liver, heart, lung, kidney, adrenal, bladder, and brain) and also in human hepatoma (HepG2) cells that are known to methylate iAs^{III} (*18*). In contrast, mRNA for this enzyme has not been found in UROtsa cells, human urinary bladder cells that do not produce methylated metabolites when exposed to iAs^{III} in culture (*12,13*).

Based on results of the *in vitro* studies, the presence of a dithiol is an essential requirement for the rat As^{III} methyltransferase activity. Thioredoxin (TRx), a small (12 kDa) protein with a pair of redox-active cysteine residues (*44*), is a likely candidate for the role of a cofactor for this enzyme in mammalian cells. In fact, TRx and DTT are equally effective in supporting the *in vitro* As^{III} methyltransferase activity (*43*). The main function of TRx in cells is the reduction of disulfide bonds in molecules of various proteins, including enzymes, cellular receptors, and transcription factors. During this reaction, the redox-active sulfhydryl groups in TRx molecule are oxidized to form a disulfide (*45*). The oxidized TRx is then reactivated in an NADPH-dependent reaction catalyzed by TRx-reductase (TR) (*44*).

The mechanism of interactions between TRx and As^{III} methyltransferase has not been examined. It is likely that TRx is involved in the reduction of the pentavalent intermediate, MAs^V. TRx may directly reduce MAs^V to MAs^{III} before the second methylation step. It may also be a donor of electrons for reduction (reactivation) of redox-active cysteinyl residues of the As^{III} methyltransferase that are responsible for MAs^V reduction. Alternatively, TRx may reduce other cysteinyl residues that are required for the catalytically active conformation of the enzyme. Notably, interactions between TRx and As^{III} methyltransferase provide a basis for a hypothetical mechanism that may play an important role in the regulation of this enzyme (Figure 6). It has been shown that MAs^{III} derivatives [$MAs^{III}O$ or $MAs^{III}I_2$] are potent inhibitors of TR (*11*), the enzyme responsible for TRx reactivation. In cell cultures exposed to iAs^{III}, inhibition of TR activity correlates with accumulation of MAs in cells (*46*). These data suggest that MAs^{III}, the intermediate formed in the course iAs methylation, is responsible for inhibition of TR activity. The inhibition of TR by MAs^{III} may result in a decreased availability of the active (reduced) form of TRx in cells, preventing further reduction of MAs^V to MAs^{III}. This hypothetical regulatory mechanism would retard the formation of MAs^{III} when the concentrations of this toxic intermediate in cells reached low micromolar values.

Table 2. Properties of As^{III} methyltransferase from rat liver.[a]

Molecular mass	42,000 Da (determined by PAGE); 41,056 Da (calculated)
Primary structure characteristics	369 amino acid residues; cysteine-rich protein (12 cysteine residues); common methyltransferase motifs, including the AdoMet binding motifs
Kinetic characteristics	K_m = 250 nM, V_{max} = 68 pmol/mg protein/min [with $MAs^{III}O$ as a substrate]; pH optimum = 9.5 (active from pH 7 to 11)
Essential co-factors	Dithiol (e.g., DTT, TRx); AdoMet (the methyl group donor)
Known inhibitor	$MAs^{III}O$ (≥ 5 μM) inhibits DMAs formation in the second methylation reaction

[a]Data from Lin et al. (*7*) and Lin and Thomas (*43*).

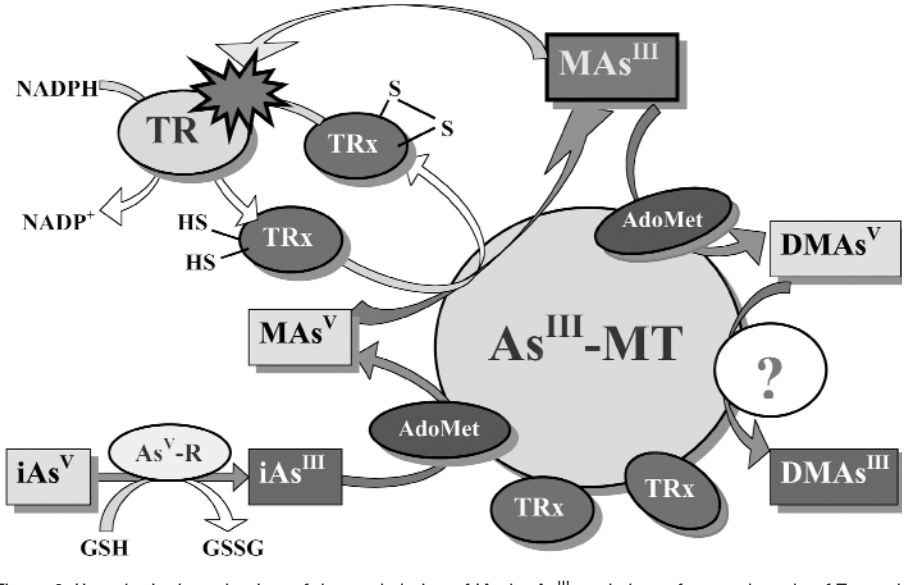

Figure 6. Hypothetical mechanism of the methylation of iAs by As^{III} methyltransferase: the role of Trx and TR. As^V-R, As^V reductase; As^{III}-MT, As^{III} methyltransferase.

Conclusions

The results of previous studies and new experimental data presented here suggest that exposures to methylated trivalent arsenicals are associated with a variety of adverse effects that have a profound impact on cell viability or proliferation. The known effects include *a*) inhibition of several key enzymes, *b*) damage to DNA structure, and *c*) activation of AP-1–dependent gene transcription. Notably, trivalent methylated arsenicals, MAs^{III} and/or $DMAs^{III}$ derivatives, are more potent than iAs^{III} in producing these effects. These findings are consistent with the concept of biomethyla-

tion being a process that potentiates toxicity and carcinogenicity of iAs.

References and Notes

1. Cullen WR, McBride BC, Reglinski J. The reaction of methylarsenicals with thiols: some biological implications. J Inorg Biochem 21:179–194 (1984).
2. Scott N, Hatlelid KM, MacKenzie NE, Carter DE. Reaction of arsenic(III) and arsenic(V) species with glutathione. Chem Res Toxicol 6:102–106 (1993).
3. Delnomdedieu M, Basti MM, Otvos JD, Thomas DJ. Reduction and binding of arsenate and dimethylarsenate by glutathione: a multinuclear magnetic resonance study. Chem Biol Interact 90:139–155 (1994).
4. Zakharyan RA, Aposhian HV. Enzymatic reduction of arsenic compounds in mammalian systems: the rate-limiting enzyme of rabbit liver arsenic biotransformation is MMA(V) reductase. Chem Res Toxico 12:1278–1283 (1999).
5. Aposhian HV, Gurzau ES, Le XC, Gurzau A, Healy SM, Lu X, Ma M, Yip L, Zakharyan RA, Maiorino RM, et al. Occurrence of monomethylarsonous acid in urine of humans exposed to inorganic arsenic. Chem Res Toxicol 13:693–697 (2000).
6. Zakharyan R, Wu Y, Bogdan GM, Aposhian HV. Enzymatic methylation of arsenic compounds: assay, partial purification, and properties of arsenite methyltransferase and monomethylarsonic acid methyltransferase from rabbit liver. Chem Res Toxicol 8:1029–1038 (1995).
7. Lin S, Shi Q, Nix B, Styblo M, Beck M, Herbin-Davis KM, Hall LL, Simeonsson JB, Thomas DJ. A novel S-adonosyl-L-methionine:arsenic(III) methyltransferase from rat liver cytosol. J Biol Chem 277:10795–10803 (2002).
8. Thomas DJ, Styblo M, Shan L. Cellular metabolism and systemic toxicity of arsenic. Toxicol Appl Pharmacol 176:127–144 (2001).
9. Styblo M, Serves SV, Cullen WR, Thomas DJ. Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. Chem Res Toxicol 10:27–33 (1997).
10. Petrick JS, Jagadish B, Mash EA, Aposhian HV. Monomethylarsonous acid (MMA(III)) and arsenite: LD(50) in hamsters and in vitro inhibition of pyruvate dehydrogenase. Chem Res Toxicol 14:651–656 (2001).
11. Lin S, Cullen WR, Thomas DJ. Methylarsenicals and arsinothiols are potent inhibitors of mouse liver thioredoxin reductase. Chem Res Toxicol 12:924–930 (1999).
12. Styblo M, Vega L, Germolec DR, Luster MI, Del Razo LM, Wang C, Cullen WR, Thomas DJ. Metabolism and toxicity of arsenicals in cultured cells. In: Arsenic Exposure and Health Effects (Chappell WR, Abernathy CO, Calderon RL, eds). Oxford:Elsevier, 1999;311–323.
13. Styblo M, Del Razo LM, Vega L, Germolec DR, LeCluyse EL, Hamilton GA, Reed W, Wang C, Cullen WR, Thomas DJ. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in human cells. Arch Toxicol 74:289–299 (2000).
14. Petrick JS, Ayala-Fierro F, Cullen WR, Carter DE, Aposhian HV. Monomethylarsonous acid (MMA(III)) is more toxic than arsenite in Chang human hepatocytes. Toxicol Appl Pharmacol 163:203–207 (2000).
15. Vega L, Styblo M, Patterson R, Cullen W, Wang C, Germolec D. Differential effects of trivalent and pentavalent arsenicals on cell proliferation and cytokine secretion in normal human epidermal keratinocytes. Toxicol Appl Pharmacol 172:225–232 (2001).
16. Mass MJ, Tennant A, Roop BC, Cullen WR, Styblo M, Thomas DJ, Kligerman AD. Methylated trivalent arsenic species are genotoxic. Chem Res Toxicol 14:355–361 (2001).
17. Le XC, Lu X, Ma M, Cullen WR, Aposhian HV, Zheng B. Speciation of key arsenic metabolic intermediates in human urine. Anal Chem 72:5172–5177 (2000).
18. Del Razo LM, Styblo M, Cullen WR, Thomas DJ. Determination of trivalent methylated arsenicals in biological matrices. Toxicol Appl Pharmacol 174:282–293 (2001).
19. Le XC, Ma M, Cullen WR, Aposhian HV, Lu X, Zheng B. Determination of monomethylarsonous acid, a key arsenic methylation intermediate, in human urine. Environ Health Perspect 108:1015–1018 (2000).
20. Mandal BK, Ogra Y, Suzuki KT. Identification of dimethylarsinous and monomethylarsonous acids in human urine of the arsenic-affected areas in West Bengal, India. Chem Res Toxicol 14:371–378 (2001).
21. Drobná Z, Styblo M. Unpublished data (2001).
22. Styblo M, Chen G-Q, Waxman S. Unpublished data (2001).
23. Styblo M, Rossman TG. Unpublished data (2000).
24. Styblo M, Blatt J. Unpublished data (2001).
25. Walton FS, Styblo M, Patel Y. Unpublished data (2001).
26. Walton FS, Styblo M. Unpublished data (2000).
27. Styblo M, Mass MJ, Kligerman AD. Unpublished data (2001).
28. Kitchin K. Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicol Appl Pharmacol 172:249–261 (2001).
29. Simeonova PP, Luster MI. Mechanisms of arsenic carcinogenicity: genetic or epigenetic mechanisms? J Environ Pathol Toxicol Oncol 19:281–286 (2000).
30. Barchowsky A, Dudek EJ, Treadwell MD, Wetterhahn KE . Arsenic induces oxidant stress and NF-κB activation in cultured aortic endothelial cells. Free Radic Biol Med 21:783–790 (1996).
31. Salazar AM, Ostrowsky-Wegman P, Menedez D, Miranda E, Garcia-Carranca A, Rojas E. Induction of p53 protein expression by sodium arsenite. Mutat Res 381:259–265 (1997).
32. Burleson FG, Simeonova PP, Germolec DR, Luster MI. Dermatotoxic chemical stimulate of *c-jun* and *c-fos* transcription and AP-1 DNA binding in human keratinocytes. Res Commun Mol Pathol Pharmacol 93:131–148 (1996).
33. Cavigelli M, Li WW, Lin A, Su B, Yoshioka K, Karin M. The tumor promoter arsenite stimulates AP-1 activity by inhibiting a JNK phosphatase. EMBO J 15:6269–6279 (1996).
34. Simeonova PP, Wang S, Toriumi W, Kommineni C, Matheson J, Unimye N, Kayama F, Harki D, Ding M, Vallyathan V, et al. Arsenic mediates cell proliferation and gene expression in the bladder epithelium: association with AP-1 transactivation. Cancer Res 60: 3445–3453 (2000).
35. Drobná Z, Jaspers I, Thomas DJ, Styblo M (submitted). Differential activation of AP-1 in human bladder epithelial cells by inorganic and methylated arsenicals. FASEB J (in press).
36. Bates MN, Smith AH, Hopenhayn-Rich C. Arsenic ingestion and internal cancers: a review. Am J Epidemiol 135:462–676 (1992).
37. Morris RD. Drinking water and cancer. Environ Health Perspect 103(suppl 8):225–231 (1995).
38. Guo HR, Chiang HS, Hu H, Lipsitz SR, Monson RR. Arsenic in drinking water and incidence of urinary cancers. Epidemiology 8:545–550 (1997).
39. Radabaugh TR, Aposhian HV. Enzymatic reduction of arsenic compounds in mammalian systems: reduction of arsenate to arsenite by human liver arsenate reductase. Chem Res Toxicol 13:26–30 (2000).
40. Zakharyan RA, Sampayo-Reyes A, Healy SM, Tsaprailis G, Board PG, Liebler DC, Aposhian HV. Human monomethylarsonic acid (MMA(V)) reductase is a member of the glutathione-S-transferase superfamily. Chem Res Toxicol 14:1051–1057 (2001).
41. Buchet JP, Lauwerys R. Study of inorganic arsenic methylation by rat in vitro: relevance for the interpretation of observations in man. Arch Toxicol 57:125–129 (1985).
42. Styblo M, Delnomdedieu M, Thomas DJ. Mono- and dimethylation of arsenic in rat liver cytosol *in vitro*. Chem-Biol Interact 99:147–164 (1996).
43. Lin S, Thomas DJ. Unpublished data (2001).
44. Holmgren A. Thioredoxin structure and mechanism: conformational changes and oxidation of the active-site sulfhydryls to a disulfide. Structure 3:239–243 (1995).
45. Holmgren A. Thioredoxin and glutaredoxin systems. J Biol Chem 264:13963–13966 (1989).