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Differential Cytotoxicity and Gene Expression in Human Liver Carcinoma ($HepG_2$) Cells Exposed to Arsenic Trioxide, and Monosodium Acid Methanearsonate (MSMA)

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Abstract: Research in our laboratory has demonstrated that a trivalent form of arsenic such as arsenic trioxide (AT) has the ability to cause significant cytotoxicity, and induction of a significant number of stress genes in human liver carcinoma cells ($HepG_2$). However, the literature also indicates that the toxicity of arsenic depends on its chemical form. To test this hypothesis, we further evaluated the cellular and molecular responses of $HepG_2$ cells following exposure to monosodium acid methanearsonate (MSMA), a pentavalent and organic form of arsenic. Cytotoxicity was evaluated using the MTT-assay for cell viability, while the gene profile assay was performed to measure the degree of gene induction in 13 different recombinant cell lines generated from a parental $HepG_2$ cell line. Cytotoxicity experiments yielded LC_{50} values of 11.9 $\pm$ 2.6 μg/mL for AT, and 257.3 $\pm$ 51.4μg/mL for MSMA; indicating that AT was about 20 times more toxic than MSMA. Exposure of $HepG_2$ cells to MSMA also resulted in a significant reduction ($p < 0.05$) in the number of stress genes induced, compared to AT. Upon MSMA exposure, only 2 (HMTIIA and HSP70) out of the 13 constructs evaluated yielded inductions to statistically significant levels ($p < 0.05$), compared to 11 (GSTYa, XRE, HMTIIA, *c-fos*, NF-kBRE, HSP70, *p*53RE, GADD153, GADD45, and GRP78) for AT. These results greatly support the hypothesis that the toxicity of arsenic compounds highly depends on their chemical forms; with the inorganic forms being more potent than the organic ones.

Keywords: Arsenic, chemical species, differential toxicity, gene expression, $HepG_2$ cells.

Introduction

Arsenic is released to the environment from natural sources as a result of natural phenomena such as erosion of mineral deposits and volcanoes, but releases from human activities such as metal smelting, coal combustion, chemical production and use, and waste disposal can lead to substantial contamination of the environment [1]. The National Academy of Science estimates that about 75,000 to 100,000 tons of arsenic are produced annually on a global scale [2]. More than 80% of arsenic compounds are used to manufacture products with agricultural applications such as insecticides, herbicides, fungicides, algicides, sheep dips, wood preservatives, dyestuffs, and medicines for the eradication of tapeworms in sheep and cattle. Arsenical drugs are still used in treating certain tropical diseases such as African sleeping sickness and amoebic dysentery, and in veterinary medicine to treat parasitic diseases, including filariasis in dogs and black head in turkeys and chickens [2]. Recently, arsenic has been used as an anticancer agent in the treatment of acute promeylocytic leukemia, and its therapeutic action has been attributed to the induction of programmed cell death (apoptosis) in leukemia cells [3]. Arsenic-containing compounds such as monosodium methanearsonate (MSMA) and disodium metahanearsonate (DMSA) have been and are still being used as to control unwanted weeds in several agricultural areas of the Mississippi Delta. MSMA is also currently used by the Mississippi Department of Transportation to control vegetation along highways and rights of way.

A very large number of people are exposed to arsenic chronically throughout the world. Exposure to arsenic occurs via the oral route (ingestion), inhalation, dermal contact, and the parenteral route to some extent [4]. For most people, the diet is the largest source of arsenic exposure, with an average intake of about 50μg per day. Intake from air, water and soil are usually much smaller, but exposure from these media may become significant in areas of arsenic contamination. People who produce or use arsenic compounds in occupations such as non-ferrous metal smelting, pesticide manufacturing and application, wood preservation, semiconductor manufacturing, or glass production can be exposed by substantially higher levels of arsenic [5,6].

It has been demonstrated that the risk of arsenic intoxication increases as a function of exposure level and duration. The National Research Council of Canada reported that 9, 16 and 44% incidence of symptoms of arsenic poisoning are observed at drinking water arsenic concentrations of 50, 50-100, and >100μg/L, respectively [7]. The frequencies of skin cancer associated with arsenic-contaminated water have been reported by the U.S. EPA as 0.26% and 2.14% at 290 and 600 μg/L, respectively. Also, cancer risks of 10^{-5}, 10^{-6}, and 10^{-7} have been estimated for drinking water containing 0.022, 0.0022, and 0.00022 μg As/L, or for eating aquatic organisms living in contaminated water containing 0.175, 0.0175, and 0.00175μg As/L [8]. In a study of cancer prevalence in patients treated with Fowler's solution (1% potassium arsenite concoction), a dose-response relationship was found between the ingested dose of Fowler's solution and the incidence of skin cancer. In patients who ingested 200 to 800 mL of Fowler's solution (1.6 to 6 g of arsenic), the prevalence of skin cancer was

5 to 10%, while patients treated with the Fowler's solution at an equivalent arsenic dose of 7.6 g had a prevalence of 20% [9]. A strong dose-response relationship between arsenic concentration in drinking water and skin cancer mortality has been documented in the endemic area of blackfoot disease in Taiwan. Similar correlations have been reported between arsenic levels in drinking water and age-adjusted mortality rates for cancers of the lung, liver, bladder, kidney, and colon [10].

The clinical manifestations of arsenic poisoning depend on the type of arsenical involved and on the duration of exposure. Symptoms of acute intoxication usually occur within 30 minutes of ingestion but may be delayed if arsenic is taken with food. In nearly all cases, the most immediate effects are severe nausea and vomiting, colicky abdominal pain, profuse diarrhea with rice stools, gastrointestinal hemorrhage and death may ensue from fluid loss and circulatory collapse. Drowsiness and confusion are often seen along the development of psychosis associated with paranoid delusions, hallucinations and delirium. Finally, seizures, coma and death, usually due to shock, may ensue [6]. Cardiac manifestations include acute cardiomyopathy, subendocardial hemorrhages, and electro-cardiographic changes. The pathological lesions described in patients with rapidly fatal arsenic intoxication are fatty degeneration of the liver, hyperemia and hemorrhages of the gastrointestinal tract, renal tubular necrosis, and demyelination of peripheral nerves [6]. Chronic exposure to arsenic affects the gastrointestinal tract, circulatory system, skin, liver kidneys, nervous system and heart. There is clear evidence from epidemiological studies that exposure to inorganic arsenic increases the risk of cancer [11]. When exposure occurs by the oral route, the main carcinogenic effect is increased risk of skin cancer. In addition to skin cancer, increased risk of other internal tumors (mainly of liver, kidney, lung, and bladder) have been reported with arsenic exposure [12,13].

Experimentally, arsenicals are fetotoxic and teratogenic in laboratory animals. The common developmental effects seen include malformations of the brain, urogenital organs, skeleton, ear, as well as small or missing eyes. Generally, these are only seen at doses that also result in maternal toxicity. *In vitro* experiments with many arsenicals have shown that they are powerful clastogens in many cell types. Tests for genotoxicity have indicated that arsenic compounds inhibit DNA repair, and induce chromosomal aberrations, and sister chromatid exchanges [4]. There are also several epidemiological studies reporting an association between exposure to inorganic arsenic and increased risk of adverse developmental effects such as congenital malformations, low birth weight, and spontaneous abortion [14].

In the environment, arsenic may be transported by wind or water erosion of small particles, or by leaching into rainfall or snowmelt. Transport and partitioning of arsenic in water depends upon the chemical form of arsenic and on interactions with other materials present [4]. Bioconcentration of arsenic occurs in aquatic organisms, primarily algae and lower invertebrates. In water, arsenic can undergo a series of transformations, including oxido-reduction reactions, ligand exchange, and biotransformation [4]. Factors influencing the fate of arsenicals include the oxido-reduction potential,

pH, metal sulfide and sulfide ion concentration, iron concentrations, temperature, salinity, distribution and composition of the biota [5].

Analyzing the toxic effects of arsenic is complicated because arsenic can exist in several different forms or valence states, and in many different inorganic and organic compounds. Several studies have indicated that the toxicity of arsenic depends on the exposure dose, frequency and duration, the biological species, age, and gender, as well as on individual susceptibilities, genetic and nutritional factors [1,4,15]. The literature also indicates that the toxicity of arsenic highly depends on its chemical form. To test this hypothesis, we evaluated the cellular and molecular responses of human liver carcinoma cells, following exposure to arsenic trioxide (inorganic arsenic) and monosodium acid methanearsonate (organic arsenic).

Materials and Methods

Chemicals and Test Media

Arsenic trioxide (As_2O_3) - Lot No. 97313-24, CASRN 1327-53-3, MW 197.84, with an active ingredient of 100% (w/v) arsenic in 10% nitric acid was purchased from Fisher Scientific in Houston, Texas. Monosodium acid methanearsonate-MSMA ($CH_3NaHAsO_3$), CASRN 2163-80-6, MW 161.96, with an active ingredient of 48.3% (w/v) was purchased from a local herbicide supplier in Jackson, Mississippi. Dulbecco's Modified Eagle's Minimal Essential Medium (DMEM) – Lot No. 1016511 was purchased from Life Technologies in Grand Island, New York.

Gene Profile and Cytotoxicity Assays

The mammalian gene profile assay (CAT-Tox) was performed for measuring differential gene expression in immortalized human liver cells. The parental cell line ($HepG_2$), and thirteen different recombinant constructs generated by creating stable transfectants of different mammalian promoter – chloramphenicol acetyltransferase (CAT) were obtained from Xenometrix, Inc. (Boulder, CO). Each construct contained a unique stress gene promoter or response element fused to the CAT reporter gene.

In the laboratory, cells were stored in liquid nitrogen until use. They were next thawed by gentle agitation of their containers (vials) for 2 minutes in a water bath at 37^{o}C. After thawing, the content of each vial was transferred to a 75 cm^2 tissue culture flask, diluted with DMEM supplemented with 10% fetal bovine serum (FBS) and 1% streptomicin and penicillin, and incubated for 24 hours at 37 ^{o}C in a 5%CO_2 incubator to allow the cells to grow, and form a monolayer in the flask. Cells grown to 80-95% confluence were washed with phosphate buffer saline (PBS), trypsinized with 10 mL of 0.25% (w/v) trypsin-0.03% (w/v) EDTA, diluted, counted, and seeded ($5x10^5$ cells/well) in two sets of 96-well microtiter tissue culture plates.

Seeded plates were incubated for 24 hrs at 37 ^{o}C in a 5%CO_2 incubator, followed by a replacement of the old medium by a fresh one containing the appropriate amount of each of the test chemicals (0,

0.3, 0.6, 1.2, 2.5, and 5 ug/mL arsenic trioxide; 0, 12.5, 25, 50, 100, and 200 ug/mL MSMA) using de-ionized water as solvent. For quality assurance/quality control purposes, positive control plates were also made using known inducers including 3-methyl cholanthrene (3-MC-10 μM) for CYP 1A1, CRE, GADD45, p53RE, and XRE; methyl methane sulphonate (MMS-100μg/mL) for GST Ya, HMTIIA, *c-fos*, HSP70, NF-kBRE, GADD153, and GRP78; and all-*trans* retinoic acid (RA-10 uM) for RARE. All chemical exposures involved the use of polypropylene 96-well microtiter plates for the purpose of chemical dilutions. A specific, constant volume (20 μL) was transferred from each well of the chemical dilution plate to the plate containing the cells to give each cell line five chemical doses and a zero control dose, each in triplicate.

Following chemical exposure, the cells were re-incubated for 48 hrs at 37^{o}C, and 5% CO_2. After the incubation period, the total protein was measured by the Bradford method, at 600 nm using a microtiter plate reader (Bio-Tek Instruments Inc.). A standard sandwich ELISA was performed and in the final step, horse radish peroxidase catalyzed a color change reaction that was measured at 405 nm [16,17].

The parental $HepG_2$ cell line was dosed in the same manner as the recombinant cell lines, and was used to perform the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] - based cell viability assay using a microtiter plate reader (Bio-Tek Instruments Inc.) with the wavelength set at 550 nm [18]. However, higher doses of arsenic trioxide (0, 10, 20, 30, and 40 μg/mL), and MSMA (0, 100, 200, 300, 400, and 500 μg/mL) were used in the initial cytotoxicity experiments to allow the determination of the chemical doses required to reduce cell viability by 50% (LD_{50}s). Subsequent experiments for gene induction were performed with subacute doses (below LD_{50}s) of arsenic trioxide (0, 0.3, 0.6, 1.25, 2.5, and 5 μg/mL), and MSMA (0, 12.5, 25, 50, 100, and 200 μg/mL).

Statistical Analysis

The transcriptional fold inductions for each recombinant cell line at each arsenic trioxide or MSMA concentration were calculated using the CAT-Tox computer software based on the optical density readings at 600 nm and 405 nm. The software also converted the 550 nm readings to cell viability percentages. Standard deviations were determined, and the *Student's t-test* values were computed to determine if there were significant differences in cell viability and gene induction in treated cells compared to the control cells. Graphs were made to illustrate the dose-response relationship with respect to cytotoxicity and gene expression.

Results

Cytotoxicity Assay

The effects of arsenic on the viability of the parental $HepG_2$ cells are shown on Figures 1 and 2. Data presented in these figures indicate a strong dose-response relationship with respect to the

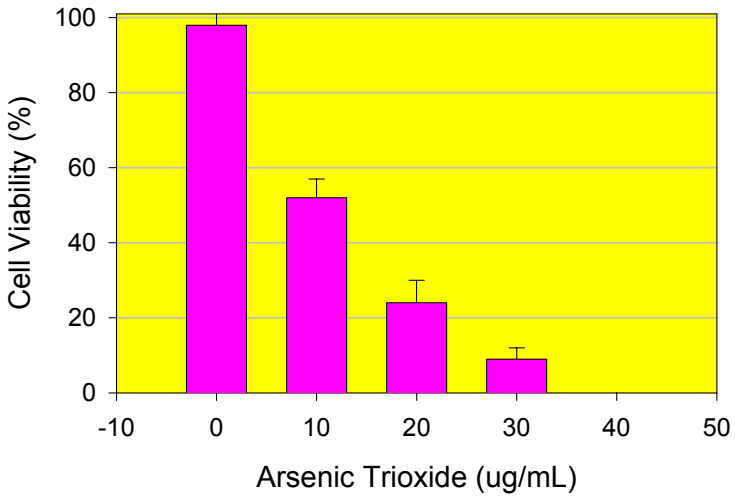

Figure 1. Cytotoxicity of arsenic thrioxide to $HepG_2$ cells.

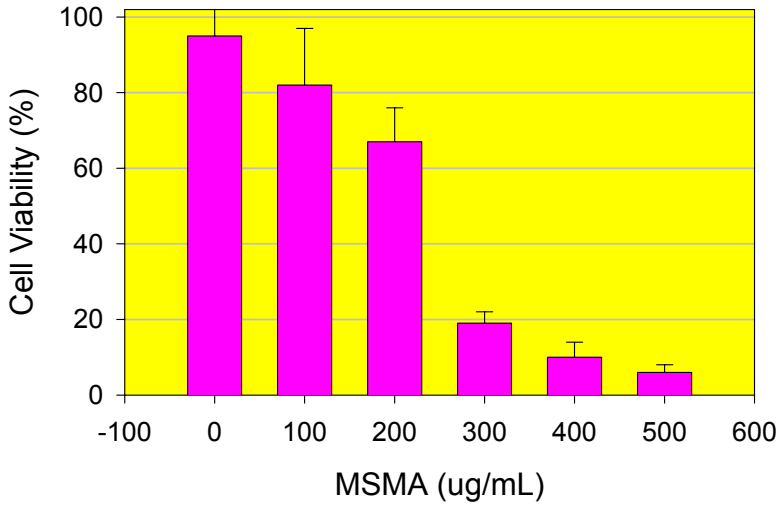

Figure 2. Cytotoxicity of monosodium acid methanearsonate (MSMA) to $HepG_2$ cells.

cytotoxicity of arsenic. Upon 48 hours of exposure, the average percentages of cell viability were 98.4±2.8%, 52.3±4.9%, 24.1±5.8%, 9.0±3.2%, and 0.0±0.0% in 0, 10, 20, 30, and 40 μg/mL arsenic trioxide, respectively (Figure 1). Similarly, the mean percentages of cell viability were 95.0±6.9%, 82.0±15.2%, 67.4±8.9%, 19.3±3.3%, 10.2±3.8%, and 6.0±2.1% in 0, 100, 200, 300, 400, and 500 μg/mL MSMA, respectively (Figure 2). LD_{50} values of 11.9 ± 2.6 μg/mL, and 257.3 ± 51.4 μg/mL were computed for arsenic trioxide, and MSMA, respectively; indicating that arsenic trioxide (inorganic form) was about 20 times more toxic than monosodium acid methanearsonate (organic form).

Gene Profile Assay

The induction levels of the metallothionein in human liver carcinoma cells exposed to arsenic are

presented in Figures 3 and 4 for arsenic trioxide, and monosodium acid methanearsonate, respectively. Data presented in these figures indicate a strong dose-response relationship with respect to arsenic induction of the HMTIIA gene promoter. The activity fold inductions of this construct were 1.0±0.0, 2.8±1.4, 12.7±3.5, 57.7±29.0, 78.0±32.3, and 132.4±55.1 in 0, 0.3, 0.6, 1.25, 2.5, and 5 µg/mL arsenic trioxide, respectively (Figure 3). Similarly, the mean fold inductions of HMTIIA were 1.0±0.0, 1.0±0.0, 6.4±5.0, and 79.0±10.0 in 0, 50, 100, and 200 µg/mL MSMA, respectively (Figure 4).

Data presented in Figures 5 and 6 also indicated a strong dose-response relationship with respect to arsenic induction of the 70-kDa heat shock protein (HSP70). Fold induction levels of 1.0±0.0, 2.4±0.6, 5.6±0.7, 16.1±21.8, 63.7±22.7, and 59.1±54.4 in 0, 0.3, 0.6, 1.25, 2.5, and 5 µg/mL arsenic trioxide, respectively (Figure 5). Similarly, the mean levels of fold induction of HSP70 were 1.0±0.0, 1.0±0.0, 3.3±3.0, and 28.0±19.0 in 0, 50, 100, and 200 µg/mL MSMA, respectively (Figure 6). Only two (HMTIIA, and HSP70) of the thirteen recombinant cell lines studied were significantly induced ($p < 0.05$) following exposure to MSMA.

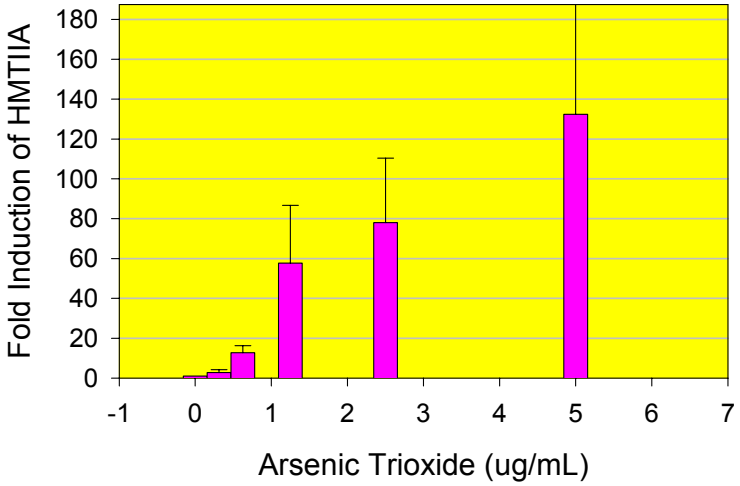

Figure 3. Fold induction of metallothionein (HMTIIA) in $HepG_2$ cells exposed to arsenic trioxide.

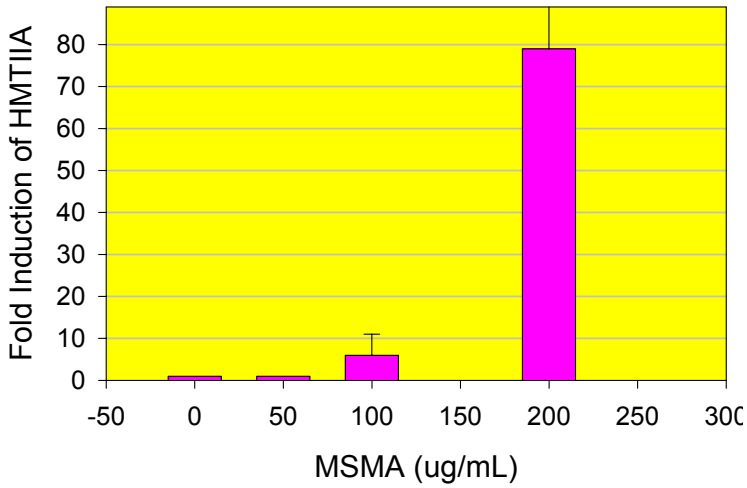

Figure 4. Fold induction of metallothionein (HMTIIA) in $HepG_2$ cells exposed toMSMA.

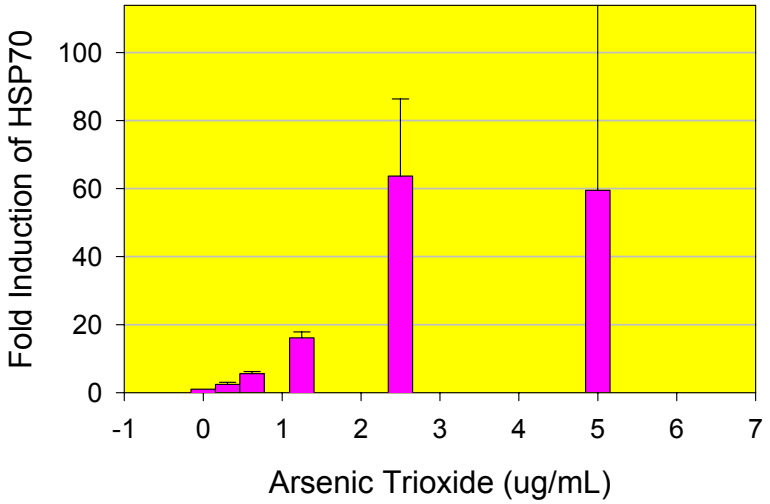

Figure 5. Fold induction of heat shock protein (HSP70) in $HepG_2$ cells exposed to arsenic thioxide.

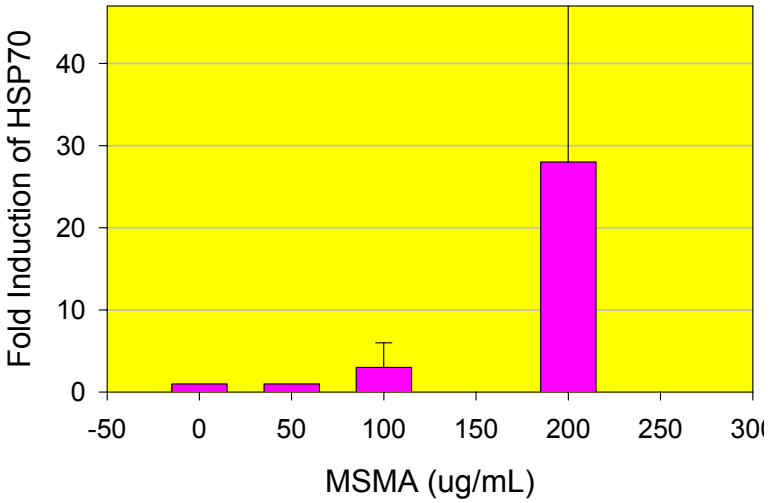

Figure 6. Fold induction of heat shock protein (HSP70) in $HepG_2$ cells exposed to MSMA.

Table 1 presents the fold inductions of other gene promoters and response elements in human liver carcinoma cells exposed to arsenic trioxide. In general, the data presented in this table showed a strong dose-response relationship with regard to the activation of the glutathione-s-transferase (GSTIIA), *c-fos*, 153-kDa growth arrest and DNA damage (GADD153), 45-kDa growth arrest and DNA damage (GADD45), and 78-kDa glucose regulated protein (GRP78) gene promoters. A similar assertion can be made for the xenotiotic response element (XRE), NF-kB, cyclic AMP, and p53 response elements (Table 1). Both arsenic trioxide and MSMA did not induce the cytochrome P450-1A1 (CYP1A1) gene promoter, and the retinoic acid response element (RARE) in a significant manner ($p > 0.05$). All QA/QC tests with positive control chemicals (3-MC, MMS, and RA) resulted in significant inductions ($p < 0.05$) of stress genes in all recombinant cell lines (data not shown).

Table 1. Fold Inductions of Other Stress Gene Promoters/Response Elements in Human Liver Carcinoma Cells Exposed to Arsenic Trioxide

Gene promoter	Arsenic trioxide dose (ug/mL)					
	0	0.3	0.6	1.25	2.5	5.0
GST Ya	1.0±0.0	1.5±0.4	3.5±0.8	10.2±1.9	21.9±4.2	26.3±8.4
XRE	1.0±0.0	1.0±0.0	1.0±0.0	1.2±0.0	1.7±0.7	2.9±1.3
C-FOS	1.0±0.0	1.1±0.4	1.6±0.7	3.9±2.0	14.5±4.2	48.2±21.7
NFk-B	1.0±0.0	1.0±0.0	1.0±0.0	1.6±0.5	3.7±1.3	5.6±5.0
CRE	1.0±0.0	1.6±0.2	1.0±0.0	1.7±0.5	1.9±0.2	3.0±0.2
p-53RE	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	3.4±0.8	51.2±10.1
GADD153	1.0±0.0	1.0±0.0	1.0±0.0	1.3±0.4	2.9±0.5	13.2±2.8
GADD45	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±1.0	2.7±0.4
GRP78	1.0±0.0	1.0±0.0	1.0±0.0	1.2±0.1	1.2±0.2	1.8±0.5

Discussion

Cytotoxicity Assay

This study demonstrated that both arsenic trioxide, and monosodium acid methanearsonate (MSMA) were acutely toxic to transformed human hepatocytes. However, arsenic trioxide appeared to be about 20 times more potent than MSMA; indicating that this inorganic form of arsenic was more hazardous to transformed human liver cells than the organic form (MSMA). These results are in agreement with those of previous studies indicating that different species of arsenic have different degrees of toxicity, with arsine and the trivalent forms causing the most injury. In addition to the exposure route, and the arsenic species involved, the toxicity also depends on the exposure dose, frequency, duration, and the biological species, as well as on individual susceptibilities, genetic and nutritional factors [4,19].

Many case reports of death in humans from ingestion of high doses of arsenic have been reported. A fatal dose of arsenic trioxide is probably in the 200 to 300 mg range, yet a dose as low as 10 to 20 mg has been life threatening, and recovery from 10 g has occurred [20]. Symptoms of acute intoxication usually occur within 30 minutes of ingestion, but may be delayed if arsenic is taken with food. In nearly all cases, the most immediate effects are severe nausea and vomiting, colicky abdominal pain, profuse diarrhea with rice stools, and gastrointestinal hemorrhage; death may ensue from fluid loss and circulatory collapse. Drowsiness and confusion are also seen along the development of psychosis, which is associated with paranoid delusions, hallucinations, and delirium. Finally, seizures, coma, and death, usually from shock may ensue. If death does not occur in the first 24 hours from irreversible circulatory insufficiency, then over the next several days death may result from hepatic or renal failure [21,22].

Previous research has pointed out that the mechanism by which arsenic exerts its toxic effect is through impairment of cellular respiration by the inhibition of various mitochondrial enzymes, and the

uncoupling of oxidative phosphorylation. Most toxicity of arsenic results from its ability to interact with sulphydryl groups of proteins and enzymes, and to substitute phosphorous in a variety of biochemical reactions [4]. Because of its high potential to cause adverse effects in exposed persons, a number of regulations and guidelines have been established for various inorganic and organic forms of arsenic by international, federal, and state agencies. The International Agency for Research on Cancer classifies arsenic in Group 1 – known human carcinogens [15,23]. The permissible limit for arsenic in drinking water has recently been lowered from 50 μg/L to 10μg/mL[24,25]. The World Health Organization=s tolerable daily intake for inorganic arsenic is 2μg/kg body weight [26]. The action level for arsenic in the air is 5μg/m^3. The permissible exposure limit-total weighted average (PEL-TWA) is 10 μg/m^3 for inorganic arsenicals, and 500 μg/m^3 for organic arsenicals [27]. The criterion for irrigation waste is 100 μg/L. Tolerances for residues in agricultural products/commodities vary from 0.35 ppm to 3.5 ppm. The oral reference dose (RfD) is $3x10^{-4}$ mg/kg/day, and the cancer potency factor is 15 $(mg/kg/day)^{-1}$ for inhalation, and 1.75 $(mg/kg/day)^{-1}$ for ingestion [4,15,19,23,28].

Gene Profile Assay

For most constructs evaluated in the CAT-Tox (L) Assay with arsenic trioxide, a dose-response relationship was recorded with respect to gene induction. For example, induction levels of 1.0±0.0, 2.8±1.4, 12.7±3.5, 57.7±29.0, 78.1±32.3, and 132.4±55.1 were recorded for HMTIIA at 0, 0.3, 0.6, 1.25, 2.5, and 5 μg/mL arsenic trioxide, respectively. Overall, eleven of the 13 tested constructs showed inductions to statistically significant levels ($p < 0.05$). At 5 μg/mL arsenic trioxide, the average levels of induction were 26.3±8.4, 2.9±1.3, 132.4±55.1, 48.2±21.7, 5.6±5.0, 59.1±53.4, 3.0±0.2, 51.2±10.1, 13.2±2.8, 2.7±0.4, 1.8±0.5, for GSTYa, XRE, HMTIIA, *c-fos*, NF*kB*RE, HSP70, CRE, *p53*RE, GADD153, GADD45, and GRP78, respectively. At this arsenic trioxide concentration (5 ug/mL), the induction of CYP 1A1 (1.3±0.1), and RARE (1.0±0.0) were not significant ($p > 0.05$).

Induction of the GSTYa promoter and XRE by arsenic trioxide indicates the potential involvement of the Phase II biotransformation pathway (conjugation) in the liver. Upregulation of GSTYa genes is controlled by the xenobiotic response element – XRE [29-31]. Inducing agents bind to the cytoplasmic ah (aryl hydrocarbon) receptor, which is present not only in the liver, but also in other extra hepatic tissues such as lung, skin and kidney. This gene responds to polycyclic aromatic hydrocarbons (PAHs) such as benzo (a) pyrene, 7- ethoxycoumarin, and 3- methylcholanthrene (3-MC). This promoter has also been reported to respond to other chemicals such as 2,3,7,8-tetrachlorlibenzo-dioxin (TCDD), polychlorinated biphenyls (PCBs) and quinone methides [16,32]. The capacity of liver cells to detoxify active compounds is correlated with the level of bio-transformation enzymes produced. Many of these enzymes including those of cytochrome P450 1A1, and the Ya subunit of glutathion-*S*-transferase are transcriptionally induced when cells are exposed to specific chemicals [16]. The GSTs are a supergene family of dimeric enzymes that catalyze the conjugation of reduced glutathione (GSH) to a variety of electrophiles including arene oxides, unsaturated carbonyls, organic halides, and other substrates [32].

GSTYa inducible transcription is controlled by two distinct response elements, the xenobiotic response element (XRE), and the anti-oxidant responsive element (ARE) [33,34].

Stimulation of the HMTIIA promoter by arsenic trioxide, and MSMA is similar to the response elicited by a variety of toxic metals such as cadmium, zinc, copper and silver [17,35-37]. Alkylating agents such as MMS have also been reported to transcriptionally induce HMTIIA [16]. It has been hypothesized that metallothioneins act as intracellular storage and concentration sites for heavy metals such as cadmium and zinc. Metallothioneins therefore mediate resistance to the toxic effects of heavy metals. They also play a role in cellular resistance to alkylating agents and radiation [37].

The induction of *p53*RE appears to be a very important factor in the pharmacology of arsenic trioxide. This tumor suppressor gene with its *p53* transcription factor shows a strong activation following exposure to arsenic trioxide. This is indicative of the potential mechanism of action of arsenic trioxide at low doses, and hence, its use as a salvage therapy for relapsed and refractory acute promyelocytic leukemia [38]. The *p53* wild type protein is involved in transcriptional activation of genes that negatively control growth and/or invasion through the *p53* response element [39]. Mutant forms of *p53* have been implicated in deregulation of gene expression in many tumor types. The *p53* response element can be upregulated by DNA damaging agents [40,41].

From a toxicological standpoint, DNA damage as a consequence of exposure to arsenic trioxide is reflected by strong inductions of *c-fos*, growth arrest DNA damage (GADD153, GADD45), and *p53*RE. In this study, the *c-fos* promoter was highly responsive to arsenic trioxide. *c-fos* forms an integral part of the AP-1 transcriptional complex along with other nuclear proto-oncogenes including *c-jun* [42,43]. The *c-fos* promoter is regulated by a variety of response elements including: 12-O-tetadecanoylphorbol 13-acetate response element (TRE), serum response element (SRE) and a cAMP response element (CRE) [44]. The cyclic AMP response element is involved in protein kinase C and protein kinase A signaling pathways [45]. Kinases are enzymes that catalyze the transfer of phosphate groups from ATP, ADP or AMP to a substance. The *c-fos* has also been implicated in the mammalian UV response pathway which appears to have an important role in the response to DNA damaging agents such as methyl methane sulfonate and 4-nitroquinoline N-oxide [46]. Activation of the GADD153 and GADD45 promoters by arsenic indicates a potential damage at the genomic level. This damage may be associated with alterations in DNA sequence, as well as conformational changes in its helical structure. Growth arrest and DNA damage promoters have been shown to respond to a variety of DNA damaging agents including UV, DNA-crosslinking, and alkylating agents [47].

MSMA did not have any significant effect on the activation the nuclear factor kappa (B site) response element (NF-kBRE). The activation of this promoter by arsenic trioxide, in combination with the *c-fos* activation, is indicative of oxidative damage by arsenic ions. The NF-kB response element is a multi-subunit transcription factor that can rapidly activate the expression of genes involved in inflammatory, immune, and acute phase responses [48]. Many different agents can activate the DNA binding activity of NF*k*B including viruses, bacterial lypolysaccharides, protein synthesis inhibitors,

cytokines including TNF-alpha, TNF-beta and interleukin-1, phorbol esters, lectins, and calcium ionophores [49].

A dose-response relationship was observed with regard to the effect of arsenic trioxide, and MSMA on the transcriptional activation of the 70-kDa heat shock protein (HSP70). Stimulation of this promoter is in line with HSP induction by many other toxicants including alkylating agents, toxic metals, and quinone methides [50]. It has been suggested that increase in HSP expression may be a direct result of alterations in protein structure through adduct formation; a secondary consequence of decreased non-protein thiol groups and formation of disulfide linkages between proteins [51]; or of release of metal ions due to alkylation of metal-sequesting proteins [52]. The 78-kDa glucose regulated protein (GRP78) is a major endoplasmic reticulum (ER) protein that functions as a chaperone. Upregulation of this promoter is associated with proteins that are malfolded because of mutagenesis, under-glycosylation, or other stress conditions in the ER [53].

Findings from this study demonstrate that arsenic trioxide and MSMA do not seem to induce transcription of retinoic acid response element (RARE). RARE is generally activated by retinoid acid analogs including all-*trans* retinoid acid and 9-*cis* retinoid acid. Retinoids have a broad range of normal biological activities in growth and differentiation [54].

Conclusions

Findings from this study indicate that: (a) acute exposure to arsenic trioxide, and monosodium acid methanearsonate significantly ($p < 0.05$) reduces the viability of human liver carcinoma ($HepG_2$) cells; and (b) arsenic trioxide was about 20 times more toxic than MSMA, supporting the hypothesis that inorganic species of arsenic are generally more hazardous than the organic ones. More importantly, this hypothesis is further supported by the data from our gene profile assay showing that exposure to arsenic trioxide transcriptionally activates up to eleven (GSTYa, XRE, HMTIIA, *c-fos*, NF-kBRE, HSP70, *p*53RE, GADD153, GADD45, and GRP78) of the thirteen gene promoters evaluated, while exposure to MSMA significantly induces only two (HMTIIA, and HSP70) of these gene products. On the basis of these findings, it can be further concluded that metallothioneins and heat shock proteins, although non specific to arsenic ions, appear to be excellent candidates for biomarkers for detecting arsenic-induced proteotoxic effects in areas of arsenic pollution. As expected, no significant inductions ($p > 0.05$) were observed for both CYP1A1, and RARE, following exposure to both arsenic trioxide, and MSMA.

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