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ARSENIC INDUCED PROLIFERATION AND SUPPRESSION OF CARCINOGENESIS CELL: A DUAL MECHANISM

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ABSTRACT

Arsenic is a naturally occurring element that exists in the environment in a number of forms, each with its own unique physical, chemical, and toxicological characteristics. The natural content of arsenic in soils, globally, ranges from 0.01 to over 600 ppm, with an average of about 2 to 20 ppm depending on the country and source of information. The arsenic is metabolite in liver and also in other parts of the body like testes, kidney and lung tissues. The metabolic pathway of inorganic arsenic involves 2 main steps of chemical reactions: reduction and oxidative methylation. Arsenic is carcinogenic to humans, and targets in particular the urinary bladder, liver, skin, lung, prostate and other internal body sites. Generation of ROS, accumulation of Ca2+, up regulation of caspase-3, down regulation of Bcl-2,deficiency of p53 are, in nut shell, the events that collectively lead to apoptosis during arsenic toxicity. Cell transformation can only be observed in cells exposed to 0.5–25 μ Marsenite.

KEYWORD: Arsenic is a naturally cells exposed to 0.5–25 μ Marsenite.

INTRODUCTION

The distribution of metals in the environment is a result of natural processes (volcanoes, erosion, spring water, bacterial activity 0 and anthropogenic activities (fossil fuel combustion, industrial process) Florea and Busselberg, 2006). Anthropogenic activities such as mining, smelting and agriculture have locally increased the levels of heavy metals such as cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), arsenic (As), and nickel (Ni) in soil up to dangerous levels for plants, animals and human beings (Sharma and Agrawal, 2005). 15. Arsenic is a naturally occurring element that exists in the environment in a number of

different forms, each with its own unique physical, chemical, and toxicological characteristics. In organic arsenic (As_i), most often in trivalent form (Arsenite, As_i^{III}) or pentavalent form (Arsenate, As_i^{V}), is the most abundant form of arsenic in nature, and is commonly present in soil, water, and food (NRC, 1999; Mok and Wai, 1994; Yan Chu, 1994). The natural content of arsenic in soils, globally, ranges from 0.01 to over 600 ppm, with an average of about 2 to 20 ppm depending on the country and source of information (Yan-Chu, 1994). Ground water in several parts of the world contains substantial amounts of arsenic, primarily due to release of naturally occurring arsenic from subsurface rock formation (Nordstrom, 2002). In contrast, methylated arsenic compounds, including mono methyl arsonic acid (MMA^{V}) and methyl arsinic acid (DMA^{V}) and their salts, are rarely detected in groundwater and, as result, human exposure to these compounds from environmental sources is expected to be minimal (NRC, 1999).[19] Chronic exposure to arsenic is a significant worldwide environmental health concern (Agency for Toxic Substances and Disease Registry 1999;National Research Council 1999). The primary route of exposure is through drinking water that has been contaminated by natural geologic sources of arsenic. In recognition of the health risks associated with chronic arsenic exposure, the U.S. Environmental Protection Agency (EPA) recently reduced its drinking water standard in regulated public water sources from 50 ppb to 10 ppb (0.67-0.13µM), but this standard does not cover private, unregulated wells (U.S. EPA,2001). Thus, drinking water arsenic exposure remains an important public health concern in many areas of the United states, such as Hampshire, were as much as half of the population acquires their water from private wells and here arsenic is naturally found at levels higher than the federal guidelines in a significant fraction of these wells (Karagas et al., 2002). Drinking water arsenic has also been implicated in impaired lung function, bronchiectasis, and increased risks of respiratory illness (Ghosh et al., 2007; Smith et al., 2006). Arsenic a lung toxicant, arsenic may be unique in its ability to increase the risk of these various lung diseases via ingestion rather than inhalation (Courtney D. Kozul et al., 2009).[20]

Natural Sources of Arsenic

Currently, Arsenic exposed is well known contaminant of water. Its exposed can be seen in whole words including Bangladesh and West Bengal-India. (Mazumder et al., 1998; Milton and Rahman, 2002; Smith and Smith, 2004). It is expected that more than 95% of the 120 million people in Bangladesh drink tube well water and more than one third of the tube well water contains arsenic above 0.05 mg/l. Several countries are also reported in High level

arsenic in drinking water such as Argentina, Australia, Chile, China, Hungary, Mexico, Peru, Taiwan, Thailand and the United States of America (Chen et al., 1992, 2004; Tseng, 1999). Several programmers have been started to provide "safe" drinking water by tube wells replacing surface water but unexpectedly underground water bring up another health problem of arsenic hazards.

Epidemiological study

Several study and a number of epidemiological data are reported, inorganic arsenic is a known carcinogen, causing tumors in skin, lung, bladder and possibly other tissues in humans exposed to high levels (Chen et al., 2003b). A number of previous studies are findings and suggested that an Arsenic caused poisonings in Bangladesh, Bengal, Thailand, Finland, Hungary, Chile, Taiwan, Vietnam, Cambodia, Mexico, Argentina and China, where geological environments are conducive to generate high amounts of arsenic compounds in ground water (Smith et al., 2000; Kayajanian, 2003; Tchounwou et al., 2003). More-over many states within the United States also have significant concentrations (up to 50 ppm) of arsenic in the groundwater (Tchounwou et al., 2003; Knobeloch et al., 2006). Other worker are reported and observed in people drinking contaminated well water in Taiwan (Lai et al., 1994; Tseng et al., 2000a, 2000b, 2002; Tseng2004) and Bangladesh (Rahman et al., 1998, 1999b), Epidemiological studies have reported associations between individual methylation patterns, specifically the proportion of MMA in urine (MMA) and the risks of several different arsenic-related diseases including bladder cancer, skin cancer, and arsenic-caused skin lesions (Del Razo et al., 1997; Hsueh et al., 1997; Yu et al., 2000; Chen et al., 2003a,b; Tseng et al., 2005; Steinmaus et al., 2006; Wu et al., 2006; Ahsan et al., 2007; Huang et al., 2007, 2008; McCarty et al., 2007; Pu et al., 2007; Lindberg et al., 2008). These data provide a highly consistent body of evidence linking methylation capacity, and specifically high % MMA, to arsenic-related disease risks.

Metabolism of arsenic

The primary site of arsenic metabolism in mammals is the liver, although there is also high methylating activity in testes, kidney, and lung tissues (Styblo et al., 2002; Vahter, 1999). Arsenic is usually found in the form of inorganic arsenate or arsenite in drinking water, depending on the pH and presence of chemical substances (Andreae, 1977; Shraim et al., 2002). The most common species of arsenic in groundwater are arsenate As(V) and arsenite As(III). Arsenic is able to undergo redox conversion between As(V) and As(III). Following

uptake from drinking water, inorganic arsenic converted into methylated arsenic forms the liver and excreted from the bladder. Thus, the bladder is the major target organ that is affected to methylated arsenic species in populations after drinking contaminated water (Tchounwou et al., 2003; Kenyon et al., 2005). The metabolic pathway of inorganic arsenic involves 2 main steps of chemical reactions: reduction and oxidative methylation (Thompson et al., 1993; Aposhian 1997; Thomas et al., 2001, 2004; Kitchin, 2001; Styblo et al., 2002; Vahter, 2002). Pentavalent arsenate is reduced to trivalent arsenite, probably mainly in the blood, before it can be further metabolized (Vahter, 2002). Arsenite is rapidly taken up by the hepatocytes than arsenate, because it is present mainly in undissociated format physiological pH, whereas arsenate is in ionized form (Vahter, 2002). It is also now known that both arsenite and arsenate are actively transported into cells by aqua glyceroporins and by phosphate transporters, respectively (Rosen, 2002). The major site for the methylation of arsenic because of its mass and the first pass effect of ingested arsenic to the liver (Thomas et al., 2001; Vahter, 2002). Previously, methylation of inorganic arsenic has always been regarded as a detoxification mechanism because MMAV and DMAV have relatively low toxicity (Yamauchi and Fowler, 1994) and are rapidly excreted in the urine (Vahter, 2002; Gebel, 2002). recent studies have confirmed the existence of trivalent intermediates and products of monomethyl arsonous acid (MMAIII) and dimethylarsinous acid (DMAIII), which are formed by reduction of MMAV and DMAV, respectively, and are more toxic than inorganic arsenite (Thomas et al., 2001; Kitchin, 2001; Styblo et al., 2002). Once inside the cell, arsenite is then oxidatively to monomethyl arsonic acid (MMAV) and dimethylarsinic acid (DMAV) (Vahter, 1999). The methylation process of arsenic takes place in the cytosol and is catalyzed by a 42-kDa protein (the methyltransferase) encoded by the cyt19 genes of mouse and human genomes and the methyl donor has been identified as S-adenosyl methionine (Thomas et al., 2004), although vitamin B_{12}, coenzyme B_{12} can also act as methyl donors (Vahter, 1999). Testes have the highest specific activity for methyl transferase in mouse, followed by kidney, liver and lung (Healy et al., 1998). But it is believed that liver is Trivalent arsenic species are stronger protein-binders than the pentavalent species and the methylation processes replace the ionizable hydroxyl groups by uncharged methyl groups, which make the arsenic species less negatively charged and able to interact directly with negatively charged molecules such as DNA at physiological pH (Kitchin, 2001). These biochemical properties of arsenic species probably explain why the trivalent methylated arsenic species are more toxic than trivalent arsenite. Because TMAIII contains no ionizable

hydroxyl groups to limit its interaction with DNA, it is postulated that this arsenic species could be very toxic and induced carcinogens is in nature (Kitchin, 2001).

Arsenic as a human carcinogen

It is evident that many metals from environmental or industrial sources are human carcinogens (Lau, A.T. et al., 2003: Chen, F. et al., 2002). Arsenic is carcinogenic to humans, and targets in particular the urinary bladder, liver, skin, lung, prostate and other internal body sites. (Chen, C.J.et al., 1990: IARC1987). Until recently, arsenic compounds were the only compounds that IARC considered to have sufficient evidence for human carcinogenicity, but inadequate evidence for animal carcinogenicity (J. Wilbourne, et al., 1986). Several epidemiological studies also implicate arsenic as a co-carcinogen in humans (reviewed in I. Hertz-Picciotoet al., 2001). In Japanese and Taiwanese populations exposed to arsenic in drinking water, associations with increased lung cancer in smokers compared to non-smokers suggest a synergy between the carcinogens (H.-Y. Chiou, et al., 1995: T. Tsuda, A et al., 1995). Global natural emissions of arsenic and arsenic compounds have been estimated to be 8000 ton each year, whereas anthropogenic emissions are about three times higher (NRC2000). Arsenic contamination of drinking water is a public health issue worldwide. Environmental exposure to arsenic is generally in the form of either arsenite (As3+) or arsenate (As5+). The former is the predominant form in drinking water from deep (anaerobic) wells, while the latter predominates under aerobic conditions. Due to carcinogenic effect of chronic arsenic expose, it has lot of concern now days. Inorganic arsenic was one of the earliest identified human carcinogens. Medical treatment of psoriasis with Fowler's solution (1% potassium arsenite) resulted in an excess of skin cancers, a finding that has lead to almost complete elimination of arsenic in human medicine. Further evidence for arsenic as a human carcinogen comes from studies of arsenic ore smelters, pesticide workers, and people exposed to arsenic-containing drinking water. In Taiwan, Chile, Argentina, Bangladesh, and Mexico, people who drink arsenic-containing drinking water develop cancers (W.P. Tseng, et al., 1968– A.H. Smith, et al., 1992).

Alteration in signaling pathways

Arsenic induced and interference in signal transduction pathway arsenic enter inside the cell through either phosphate transport proteins or aqua glyceroporin simple diffusion, and get be metabolized in some cell, such as hepatocytes, major pathway affected include the tyrosine

phosphorylation system, mitogen activated protein kinases (MAPKs) and transcription factor families such as NF-kB and AP-1 (Qian, Y.et al., 2003. Tapio, S et al., 2006).

Arsenic Induces Cell Transformation

Much of the lack of progress in determining mechanisms explaining the role of arsenic as a carcinogen or as a chemotherapeutic agent has been attributed to the availability of valid and reproducible animal models. To study whether arsenite induces cell transformation, we exposed JB6Cl41 cells to arsenite in soft agar. Anchorage-independent colonies were observed in the eighth week after arsenite exposure. Cell transformation can only be observed in cells exposed to 0.5–25 μMarsenite, whereas no transformed colonies were observed at higher concentrations of arsenite (50–100 μM) because of the toxicity of arsenic (Huang et al. 1999a, 1999b). It has been suggested that arsenic might act as a co-carcinogen or a promoter in carcino-genesis by mode of action studies (T. G. Rossman et al., 2004). Oxidative stress, altered growth factors and chromosomal abnormality and may contribute to arsenic carcinogenesis (C.Kojima, et al., 2009 K. T. Kitchin et al., 2001 P. B.Tchounwou, et al., 2003).

Arsenic tends to binds to the thio-group (-SH) of proteins, targeting regulatory or structural proteins (E. T. Snow 1992, M. Lu et al., 2008). Approximately 200 proteins could be targeted by the bindings and interactions of arsenic-thio group (C. O. Abernathy, et al., 2001). Among these proteins, the proto-oncogene c-Jun is well investigated. By binding to thio-groups, arsenic can block Jun N-terminal kinase (JNK) phosphatase activity, resulting in an over activation of JNK, which activates proto-oncogene c-Jun, inducing c-Jun/c-Fos (AP-1) mediated gene up regulations (D.Zhang, et al., 2009 M. Cavigelli et al.,1996). These up regulatedgenes include cell cycle regulation, and apoptotic signaling, all of which are strongly linked to arsenic carcinogenesis.

Genomic instability and chromosome abnormalities is another possible mechanism in arsenic carcinogenesis is through Arsenic is repeatedly induces chromosome abnormalities and aberrant sister chromatid exchanges (P. Ghosh, et al., 2007 J. Mahata, etal., 2003). These chromosomal abnormalities were highly associated with arsenic-induced oxidative DNA damages (M. Matsui et al., 1999 K.Yamanaka and S. Okada 1994) and might link to arsenic carcinogenesis. The one more possible etiological factor leading to arsenic carcinogenesis is through abnormal DNA repair and epi-gentic regulations. Arsenic was able to inhibit DNA repair systems in the steps of nucleotide excision repair (X. J. Qin, et al., 2003 H. K.

Hamadeh et al., 2002), DNA ligase III activity, DNA base excision re-pair(J. H. Li and T. G. Rossman 1989 Y. Hu, et al., 1998) and DNA strand break rejoining (S. Lynn, et al.,1997).

Arsenic as a Apoptosis inducer

Apoptosis or programmed cell death plays a key role in the development and homeostasis of the different system (Boise et al., 1995). Apoptosis is a critical cellular response to maintain normal cell development and proper function of multi cellular organism. Studies are also able two major pathways of apoptosis the death receptor pathway of apoptosis, and the mitochondrial mediated cytotoxicity (Cohen et al., 1992) Although apoptosis is very important in the homeostasis system, this type of cell death can also be associated with several diseases. An abnormal resistance to the induction of apoptosis has been observed in different neoplastic or autoimmune conditions. In contrast, enhanced apoptosis has been foundin disorders such as AIDS, a plasticanemia and neurodegenerative disorders (Alison and Sarraf, 1995). Abnormalities in apoptosis of cells, which may result in, autoimmune disease or malignancy (Ling etal., 2002).Several studies are reported, two major pathways are implicated in apoptosis: the death receptor pathway and the mitochondrial pathway (Gupta, 2002; Green and Reed, 1998). Oxidative stress, radiations and chemotherapeutic agents may trigger the mitochondrial pathway. Increased permeability of the mitochondrial membrane during apoptosis triggers the release of cytochrome-*c* into the cytoplasm is a critical step, which is followed by the activation of different caspases like enzyme caspase 9 and caspase 3 (Sen etal., 2004). Cytochrome *c* binds to the adapter protein apoptotic protease-activating factor (Apaf-1); in presence of ATP and then unites with procaspase 9 to form an apoptosome. This leads to the autolytic activation of procaspase 9 to active caspase 9, which in turn cleaves and activates downstream procaspase 3 to active caspase 3. At this stage, several different signaling pathways converge, causing cleavage of multiple downstream substrates. This results in several morphological and biochemical changes leading to apoptosis of the cell (Hengartner, 2000).

Other studies are reported highly relevant information on arsenic-induced apoptosis. Generation of ROS, accumulation of Ca 2+, up regulation of caspase-3, down regulation of Bcl-2,deficiency of p53 are, in nut shell, the events that collectively lead to apoptosis during arsenic toxicity. Therefore, role of oxidative stress and associated biochemical events in apoptosis deserve special mention. It was confirmed thatAs2O3-induced apoptosis through

the oxidative pathway Depleting intracellular GSH (Tang Q et al., 2006 Flora AM, et al., 2005).

Arsenic-mediated ROS-induced apoptosis

Apoptosis is a critical cellular response to maintain normal cell development and proper function of multi cellular organism. Studies are also able two major pathways of apoptosis the death receptor pathway of apoptosis and the mitochondrial pathway (95) Reactive oxygen species (ROS) is found to play a critical role in induction of apoptosis under both physiological and pathological conditions. Interestingly, mitochondria are both the source and target of ROS. Arsenic exerts its toxicity by generation of ROS (Hei et al., 1998; Kitchin and Ahmad, 2003; Liu et al., 2003; Das et al., 2005).

ROS, produced mainly in the mitochondria lead to the free radical attack of the membrane phospholipids leading to mitochondrial membrane disruption (Gupta et al., 2003). Mitochondria are the primary site of arsenic intoxication either indirectly via ROS accumulation or directly condensing mitochondrial matrix and opening of permeability transition pores by virtue of its thiol-oxidizing properity, in either case, arsenic induced ROS induced and further Disruption of the MMP bring morphological changes and cause loss of mitochondrial organization (Santra et al., 2007 Banerjee et al, 2008) which triggers a cascade of events such as release of cytrochrome-c, activation of apoptosis protein (Bax, Bad,) and down regulation of Bcl2, leading to apoptosis (Pulido et al., 2003 Mishra et al., 2008) According to another school of thought, mitochondrial membrane disruption may occur first which in turn may be responsible for increased ROS generation from it, which of these two events occurs first, is highly debatable. However, the disruption of the mitochondrial membrane results in the increased release of cytochrome-*c* in the cytosol leading to the activation of caspase 3. Caspase 3 activation leads to DNA breakage, nuclear chromatin condensation and finally causes apoptosis of the cell. In our study, a significantly higher level of ROS production, enhanced loss of mitochondrial membrane potential, increased levels of cytochrome-*c* in the cytosol and increased activation of caspases (both 9 and 3). These results are in agreement with the observations of previous *in vitro* study (Finucane et al., 1999). Similar results were found in the *in vitro* work of Gupta et al. (2003), where during apoptosis, the cleavage patterns of genomic DNA are typical of intra nucleosomal DNA digestion by an endogenous nuclease, which is considered as a hallmark of apoptotic cell death. Our results show arsenic-induced formation of DNA ladder in the PBMC of the arsenic exposed skin

lesion individuals. There is no ladder formation in case of the arsenic unexposed individuals (controls). Induction of apoptosis is also associated with the morphological alterations of the nucleus as is evident from co focal microscopic results. These observations indicate that chronic arsenic exposure causes significant DNA damage in the exposed individuals that may ultimately lead to increased apoptosis in them. Again, the release of cytochrome *c* and apoptosis inducing factor (AIF) is finely tuned by Bcl-2 family proteins in either of the following two ways: according to one possible mechanism, Bax present in the cytoplasm is translocated to the mitochondrial membrane, where it undergoes conformational changes assisted by Bid. The binding of Bax to the outer membrane (OM) causes it`s *in situ* multimerization, whereby PTP is gated and cytochrome *c* is released. Bcl-2 and Bcl-xL inhibit conformational changes in Bax and therefore inhibit release of cytochrome *c* and apoptosis. Kuwana et al. (2002) suggested the other possible mechanism of the release of cytochrome *c* into the cytosol. They have demonstrated that Bid, Bax, and lipid form supramolecular openings in the OM membrane and that during apoptosis mitochondrial protein release through supramolecular openings in the OM is promoted by Bid/Bax and directly inhibited by Bcl-xL.

Carcinogenesis Consequence Cell death

A model of arsenic action based on our data is presented. Outcome depends on the dosage of arsenite exposed, which can either lead to cell proliferation through the ERK pathway or apoptosis through the JNK pathway. The key role of mitochondria in apoptosis was further hypothesized by Richter et al., 1993 According to his viewpoint, uncontrolled production of oxygen radicals, a common step in many models of apoptosis (J.Chandra et al., 200), stimulates Ca2+ release from mitochondria, followed by Ca2+ cycling. Subsequently, Ca2+ cycling causes mitochondrial uncoupling, drop of $\Delta\psi$, ATP depletion, massive disturbance of cellular Ca2+ homeostasis, and a direct stimulation of Ca2+-dependent endonuclease(s). The importance of Ca2+ in apoptosis was reviewed in detail recently (S.Orrenius et al., 2003). To explain the protective functions of Bcl-2, a model was proposed in which Bcl-2 regulates an antioxidant pathway at sites of free radical generation (D.M. Hockenbery, et al.,1993), although the detailed investigation of the location of Bcl-2 revealed that it is localized not in the inner but in the outer mitochondrial membrane (M. Nakai, et al., 1993). Currently, it is widely accepted that mitochondria play a key role in the regulation of apoptosis (S. Orrenius, et al., 2004). Specifically, the release of different pro-apoptotic proteins that are normally

present in the inter membrane space of these organelles has been observed during the early stages of apoptotic cell death (J. Cai, et al.,1998 D.R. Green, et al.,1998).

CONCLUSIONS

There appears to be no unifying mechanism by which Metals act to alter the apoptotic process. However, understanding the mechanisms of apoptosis is caused by Carcinogenic and non-carcinogenic metals may open a venues to manipulate cells during metal toxicity. In an analogous fashion, enhance the apoptosis may destroy specific critical cell population so may allow damaged cells to escape appropriate destruction. Suppression of apoptosis may facilitate aberrant cell accumulation which may be critical step in the pathogenesis of Malignancy or autoimmunity.

In this study, we are find a higher concentration of arsenic appears to prevent cell transformation by inducing apoptosis. We showed that treatment of cells with a relatively higher concentration (200 μM) of arsenite or arsenate resulted in apoptosis by 44.5 and 61.5%, respectively (Chen et al. 2000b).

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