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Induction of glutathione synthesis in human hepatocytes by acute and chronic arsenic exposure: Differential roles of mitogen-activated protein kinases

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ABSTRACT

Glutathione (GSH) is a vital component of antioxidant defense which protects cells from toxic insults. Previously we found intracellular GSH was involved in cell resistance against arsenic-induced cytotoxicity. However, molecular mechanisms of GSH homeostasis during arsenic exposure are largely undefined. Here, we investigated roles of mitogen-activated protein kinases (MAPKs) in GSH synthesis pathway with two arsenic exposure strategies by using Chang human hepatocytes. In one strategy, acute arsenic exposure (20 μM, 24 h) was applied, as MAPK signaling is generally considered to be transient. In the other one, chronic arsenic exposure (500 nM, 20 weeks) was applied, which mimicked the general human exposure to arsenic. We found that acute arsenic exposure activated extracellular signal-regulated 1/2 kinases (ERK1/2) and c-Jun N-terminal kinase (JNK) in parallel with increased transcription and nuclear translocation of factor-erythroid 2-related factor 2 (NRF2) and enhanced expression of γ-glutamyl cysteine ligase catalytic subunit (GCLC), resulting in elevated intracellular GSH levels. Specific ERK inhibitor abolished arsenic-induced NRF2 nuclear translocation and GSH synthesis. During chronic arsenic exposure which induced a malignant cellular phenotype, continuous p38 activation and NRF2 nuclear translocation were observed with enhanced GSH synthesis. Specific p38 inhibitor attenuated arsenic-enhanced GSH synthesis without changing NRF2 nuclear translocation. Taken together, our results indicate MAPK pathways play an important role in cellular GSH homeostasis in response to arsenic. However, the specific activation of certain MAPK is different between acute and chronic arsenic exposure. Furthermore, it appears that during chronic arsenic exposure, GSH synthesis is regulated by p38 at least in part independent of NRF2.

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1. Introduction

Arsenic is a natural metalloid element found in a low concentration in almost every part of the environment. In the public mind, arsenic is usually recognized as a notorious toxicant though it has also been used in medical therapy, such as the treatment of acute promyelocytic leukemia. Humans are mainly exposed to arsenic by occupation, environment and medication. Acute arsenic poisoning is rare in the modern world, and occurs mostly in homicide, suicide or accident (Lech and Trela, 2005). Acute poisoning can cause severe systemic toxicity and death. Common clinical features include abdominal pain, nausea, vomiting, diarrhea, intense thirst, muscle cramps and a postponed peripheral neuropathy (Lech and Trela, 2005; Xu et al., 2008a). On the other hand, chronic arsenic poisoning can be seen relatively more often, of which drinking arsenic-contaminated groundwater is a major threat to the public health. Millions of people worldwide are chronically exposed to arsenic in drinking water. Recently, it has been estimated that

Abbreviations: A-As, acute arsenic-exposed; ANOVA, analysis of variance; ARE, antioxidant responsive element; BHA, butylated hydroxyanisole; BSO, buthionine sulfoximine; C-As, chronic arsenic-exposed; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); ERK1/2, extracellular signal-regulated kinases 1/2; FBS, fetal bovine serum; GCL, γ-glutamyl cysteine ligase; GCLC, γ-glutamyl cysteine ligase catalytic subunit; GCLM, γ-glutamyl cysteine ligase modulatory subunit; GSH, glutathione; JNK/SAPK, c-Jun N-terminal kinase/stress-activated protein kinase; KEAP-1, kelch-like ECH-associated protein 1; MAPKs, mitogen-activated protein kinases; MMP, metalloproteinase; NAC, *N*-acetyl-L-cysteine; NRF2, nuclear factor-erythroid 2-related factor 2; ROS, reactive oxygen species; SAM, *S*-adenosylmethionine; *t*BHQ, *t*-butylhydroquinone; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction.

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19.6 million people in China are being exposed to high levels of arsenic (>10 μg/L) via drinking groundwater according to a new statistical risk model (Rodriguez-Lado et al., 2013). Typical clinical features of chronic arsenic poisoning are skin lesions, including hyperkeratosis, pigmentation and skin cancers (IARC, 2012). In addition, chronic exposure to arsenic has been associated with cancers of other organs (e.g., the liver, lung and bladder) and diseases of other systems (e.g., cardiovascular dysfunction, respiratory symptoms, neurological defects, diabetes, and reproductive issues) (Bolt, 2012; IARC, 2012).

Oxidative stress, an imbalance between oxidant production and antioxidant defense that leads to cellular macromolecular damage and dysfunction, plays a critical role in arsenic toxicity. Substantial data suggest that arsenic can participate in the cellular oxidation–reduction reactions leading to the generation of excessive reactive oxygen species (ROS) and nitrogen species (Valko et al., 2005). In epidemiological studies, arsenic exposure was found to decrease antioxidants and increase lipid peroxidation and oxidative DNA damage (Pi et al., 2002; Pineda-Zavaleta et al., 2004; Xu et al., 2008a,b). The toxic effects of arsenic are closely related to the antioxidant defense in the body. Glutathione (GSH), a tripeptide composed of cysteine, glutamic acid and glycine, is a vital component of antioxidant defense mechanism (Forman et al., 2009). It also serves as a cofactor for conjugation of many xenobiotics catalyzed by glutathione S-transferase. GSH exerts complex roles during arsenic exposure by: (1) helping to detoxify arsenic-induced ROS directly or indirectly as a substrate of glutathione peroxidase; (2) serving as a reducing agent for the reduction of arsenate to arsenite (Scott et al., 1993); and (3) forming arsenic–glutathione conjugates which are required for arsenic excretion from the cells and the substrates for *S*-adenosylmethionine (SAM)-dependent methyltransferase, a key enzyme in arsenic methylation (Hayakawa et al., 2005; Watanabe and Hirano, 2013). Previously, we have observed that intracellular GSH of Chang human hepatocytes was involved in cell resistance to arsenic-induced cytotoxicity and apoptosis (Wang et al., 2009). However, the molecular mechanism of GSH regulation in the cell with arsenic exposure remains elusive.

Nuclear factor-erythroid 2-related factor 2 (NRF2) is an inducible transcription factor that can be activated in response to oxidative stress (Kobayashi et al., 2006). Under normal conditions, NRF2 is repressed by the inhibitory factor Kelch-like ECH-associated protein 1 (KEAP-1) in the cytoplasm (Itoh et al., 1999). Oxidative insult or electrophilic stress, such as arsenic exposure, can disassociate NFR2 from KEAP1 (Kobayashi et al., 2006). Then NFR2 translocates and accumulates into the nucleus where it activates antioxidant responsive element (ARE)-mediated transcription of cyto-protective genes including γ-glutamyl cysteine ligase (GCL) (Solis et al., 2002). The mitogen-activated protein kinases (MAPKs) are crucial signaling pathways in cell differentiation, proliferation and cell death (Blenis, 1993) in response to various factors such as oxidative stressors (Genestra, 2007). There are three distinct members of MAPKs in mammals: extracellular signal-regulated kinases 1/2 (ERK1/2), c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and p38 (Genestra, 2007). Each member may have different activators and substrates (Kusuhara et al., 1998). Abundant evidence has demonstrated that MAPK pathways are also involved in the regulation of NRF2-ARE axis (Correa et al., 2012; Kachadourian et al., 2011; Kong et al., 2001). Recently, a model has been proposed that xenobiotics, e.g., butylated hydroxyanisole (BHA), de-methylated metabolite *t*-butylhydroquinone (*t*BHQ), green tea polyphenols, sulforaphane and etc., activate the MAPK pathway and lead to the activation of NRF2/MAF hetero-dimers on ARE with the subsequent induction of cellular defense/detoxifying genes, resulting in protection of cells against toxic insults (Kong et al., 2001). In astrocyte-rich cultures, ARE-mediated transcription was enhanced by inactivation of p38 and attenuated by inhibitors of ERK1/2 and JNK (Correa et al., 2012). It has been observed in mouse embryonic fibroblasts that genetic or pharmacological inhibition of p38 suppressed NRF2 activation (Rubio et al., 2014).

Inorganic arsenic undergoes biomethylation in humans which is proven to be closely related to arsenic excretion and arsenic toxicity (Gebel, 2002; Tseng, 2009). The liver is considered to be a primary organ of arsenic biomethylation, as well as a main target of arsenic toxicity. In our previous study, we found that Chang human hepatocytes had an efficient arsenic methylating capacity (Wang et al., 2009). In addition, intracellular GSH levels were involved in cell resistance to arsenic-induced cytotoxicity and apoptosis in this cell model (Wang et al., 2009). Thus, in the present study, the role of MAPK pathways in GSH synthesis was investigated in Chang human hepatocytes during arsenic exposure. As a key transcription factor in oxidative defense mechanism and upstream regulator of GSH synthesis, NRF2 was also checked. Currently, most studies on signaling pathways are conducted in acute exposure experiments. However, it is quite more often to see chronic arsenic exposure in real life. Since acute and chronic arsenic exposures are different in the exposed doses, time and clinical features, varied molecular mechanisms may be involved. Thus, we conducted our studies with acute and chronic arsenic exposure strategies to compare the molecular events mentioned above.

2. Materials and methods

2.1. Cell culture and treatment

Chang human hepatocytes were obtained from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China) and cultured as previously described (Wang et al., 2009; Xu et al., 2010). Briefly, cells were grown in RPMI 1640 medium (Gibco, Rockville, MD) supplemented with 10% of fetal bovine serum (FBS), 2.0 mg/ml of sodium bicarbonate, and antibiotics (100 U/ml of penicillin and 100 μg/ml of streptomycin) under a humidified atmosphere of 5% CO_2 to 95% air at 37 °C.

In acute arsenic exposure experiments, cells at 80% confluence were exposed to 20 μM of sodium arsenite (Sigma, St. Louis, MO), which is within the range of internal arsenic exposure levels of acute arsenic poisoning in humans (Tournel et al., 2011), for up to 24 h in 25 cm^2 culture flasks. Cell viability was above 80% under these conditions. Pretreatment of 5 mM of *N*-acetyl-L-cysteine (NAC, Sigma, St. Louis, MO) or 0.5 mM of buthionine sulfoximine (BSO, Sigma, St. Louis, MO) for 1 h, or 20 μM of a specific MAPK inhibitor (MEK1/2, PD98059; JNK, SP600125; p38, SB203580, all from Sigma, St. Louis, MO) for 2 h was applied where indicated. In chronic arsenic exposure experiments, cells were exposed to 500 nM of sodium arsenite for up to 20 weeks (wks). Passage-matched cells were used as control. In some assays, chronic arsenic-exposed (C-As) cells were treated with NAC, BSO or a specific MAPK inhibitor as mentioned in acute arsenic exposure experiment except the treatment was applied after 20 wks of arsenic exposure.

2.2. Cell viability assay

Untreated and C-As cells were seeded at 1.0×10^5 cells per well in 96-well plates and incubated at 37 °C with 5% CO_2. After 24 h, cells were treated with NAC, BSO, or a specific MAPK inhibitor as indicated and then exposed to 0–200 μM of sodium arsenite for 24 h, at which point cell viability was measured using a CellTiter 96 AQueous One Solution Cell Proliferation Assay kit (Promega, Madison, WI) according to the manufacturer's instruction. This method measures the production of formazan conversed from

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