Environmental Pollution 234 (2018) 590–600

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Cortex and hippocampus DNA epigenetic response to a long-term arsenic exposure via drinking water☆

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ARTICLE INFO

Article history:
Received 21 September 2017
Received in revised form
3 November 2017
Accepted 25 November 2017

Keywords:
Arsenic
DNA epigenetics
Oxidative stress
DNA methyltransferases
Ten–eleven translocations

ABSTRACT

The neurotoxicity of arsenic is a serious health problem, especially for children. DNA epigenetic change may be an important pathogenic mechanism, but the molecular pathway remains obscure. In this study, the weaned male Sprague-Dawly (SD) rats were treated with arsenic trioxide via drinking water for 6 months, simulating real developmental exposure situation of children. Arsenic exposure impaired the cognitive abilities, and altered the expression of neuronal activity-regulated genes. Total arsenic concentrations of cortex and hippocampus tissues were significantly increased in a dose-dependent manner. The reduction in 5-methylcytosine (5 mC) and 5-hydroxymethylcytosine (5hmC) levels as well as the down-regulation of DNA methyltransferases (DNMTs) and ten–eleven translocations (TETs) expression suggested that DNA methylation/demethylation processes were significantly suppressed in brain tissues. S-adenosylmethionine (SAM) level wasn't changed, but the expression of the important indicators of oxidative/anti-oxidative balance and tricarboxylic acid (TCA) cycle was significantly deregulated. Overall, arsenic can disrupt oxidative/anti-oxidative balance, further inhibit TETs expression through TCA cycle and alpha-ketoglutarate (α-KG) pathway, and consequently cause DNA methylation/demethylation disruption. The present study implies oxidative stress but not SAM depletion may lead to DNA epigenetic alteration and arsenic neurotoxicity.

1. Introduction

Arsenic pollution is a serious environmental issue worldwide (Rodriguez-Lado et al., 2013). Peripheral neuropathy is commonly observed in humans chronically exposed to inorganic arsenic (iAs) contaminated drinking water (Vahidnia et al., 2007). Besides, iAs is tightly associated with various adverse effects in central nervous system (CNS) (Järup, 2003). Epidemiological investigations suggested that chronic arsenic exposure could damage neurobehavioral function, impair verbal intelligence quotient (IQ) and long-term memory and switching attention in children and adolescents (Ehrenstein et al., 2007; Wasserman et al., 2007; Rosado et al., 2007; Hamadani et al., 2011). The alterations in memory and attention processes have also been reported in adults acutely exposed to iAs (O'Bryant et al., 2011; Carroll et al., 2017). Similarly, animal studies also showed iAs exposure could cause neurobehavioral changes, such as the alterations in learning and memory (Tyler and Allan, 2013).

Epigenetic dysregulation plays an important role in arsenic toxicity. Increasing evidences show that arsenic exposure may alter DNA methylation levels (Reichard et al., 2007; Reichard and Puga, 2010; Ren et al., 2011; Lambrou et al., 2012). More recently, an in-vitro study demonstrated short-term acute arsenic exposure might induce epigenetic DNA reprogramming and delayed epigenetic effects even after treatment removal (Mauro et al., 2016). The regulatory processes of DNA methylation dynamics influence CNS function and are altered in neurological disorders (Feng and Fan, 2009). However, few are known about the influence of arsenic exposure on DNA demethylation process in brains. 5-

☆ This paper has been recommended for acceptance by David Carpenter.

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https://doi.org/10.1016/j.envpol.2017.11.083
0269-7491/

hydroxymethylcytosine (5hmC) is thought as the sixth DNA base, accounting for ~40% of modified cytosine in brain (Szulwach et al., 2011). 5-methylcytosine (5 mC) can be enzymatically modified to 5hmC by ten–eleven translocation (TET) family enzymes through Fe(II)-α-KG–dependent hydroxylation, which presents an intriguing DNA demethylation mechanism in brains (Chia et al., 2011). Recently, we observed the organ-specific alterations of 5hmC in the rats exposed to arsenic-contaminated drinking water (Zhang et al., 2014). These facts yield a new perspective that arsenic induced 5 mC-dependent regulatory disruption in brains.

To date, the epigenetic mechanism of arsenic-mediated neurotoxicity remains obscure. S-adenosylmethionine (SAM) works as methyl donor for both arsenic methylation and DNA methylation processes. The competition for SAM is proposed to be an effect of arsenic exposure that leads to a significant reduction in genomic DNA methylation. Liver is the main site for arsenic methylation, and extensive hepatic SAM depletion by arsenic metabolism was reported to suppress the activities of DNA methyltransferases (DNMTs) and other cellular methyltransferases (Hoffman et al., 1980; Caudill et al., 2001). However, it remains a debate whether arsenic exposure induces significant SAM deficiency in brain tissues. iAs is difficult to cross the blood-brain barrier (BBB). The iAs level in cerebrospinal fluid (CSF) reached only 17.7% of the corresponding levels in plasma (Au et al., 2008). In a recent study, for life-long arsenic exposed rats through drinking water (3 ppm), SAM was severely decreased in liver, but no change was observed in the brain (Ríos et al., 2012). Therefore, beyond the classic model of SAM deficiency, there must be another potential epigenetic pathway responsible for arsenic neurotoxicity.

Oxidative stress is a widely documented mechanism of arsenic toxicity. Brain consumes 80% of the oxygen, so it is more susceptible to oxidative damage when compared with other organs (Dringen et al., 2000; Bharath et al., 2002). Arsenic could exert its effects on gene regulation by the generation of reactive oxygen species (ROS) in brains (Xi et al., 2010; Jomova and Valko, 2011; Herrera et al., 2013). In this study, we hypothesized that arsenic-induced ROS disrupts DNA methylation and demethylation dynamics through tricarboxylic acid (TCA) cycle and alpha-ketoglutarate (α-KG) pathway in brains (Fig. 1). Sirtuin3 (SirT3) is a nicotinamide adenine dinucleotide (NAD+) dependent protein deacetylase in mammals, which was recently found to activate isocitrate dehydrogenase 2 (IDH2) through deacetylation (Chia et al., 2011). TETs are α-KG-dependent dioxygenases that can catalyze the oxidation of 5 mC to 5hmC and then may lead to a global DNA demethylation, while the excessive oxidative stress may affect the activities of SirT3 and IDH in the TCA cycle, thereby disrupt the conversion process between isocitric acid and α-KG in brain.

When the exposure occurs in the developmental stages, the nervous system is more susceptible to toxic agents. So far, there has been little documentation examining the arsenic-induced brain DNA methylation changes during childhood. In this study, we mimicked the developmental exposure situation by treating the weaned rats with arsenic-contaminated drinking water for six months. We investigated the effects of arsenic on spatial learning ability and related gene expression. Furthermore, we investigated the response of DNA methylation/demethylation processes in brain tissues (i.e. cortex and hippocampus), and the molecular mechanisms were tested on the above-mentioned hypothesis.

2. Materials and methods

2.1. Animals treatment

Thirty weaned male Sprague-Dawly rats (weight 75 ± 7 g) were obtained from Shanghai Laboratory Animal Center, China. All animals were maintained under a 12 h light–dark cycle and had free access to the water and pellet. After the acclimatization, the rats were randomly divided into the control group, 7.5 μM and 200 μM arsenic trioxide (ATO)- treated groups. The doses were selected according to environmental arsenic levels of ground water in heavily polluted areas. An estimated 100 million people worldwide are exposed to the ppm range of arsenic via drinking water (Tyler and Allan, 2014). In Blackfoot disease–affected areas of Taiwan, arsenic concentrations in drinking water reached 15 μM (Heydorn, 1970). The doses used were also comparable or lower than those reported in previous toxicological studies (Luo et al., 2009; Tyler and Allan, 2014). The rats were exposed to ATO for six months. In the last week of exposure, Morris water maze (MWM) test was conducted to evaluate the alteration of the learning and memory ability. After the test, the rats were sacrificed by decapitation. Cortex and hippocampus were dissected out and stored at −80 °C for the further analysis.

2.2. Morris water maze test

The rats were trained on the hidden platform version of MWM (the details were shown in Supplemental Material A1). The time that the rats spent to find the submerged platform was recorded.

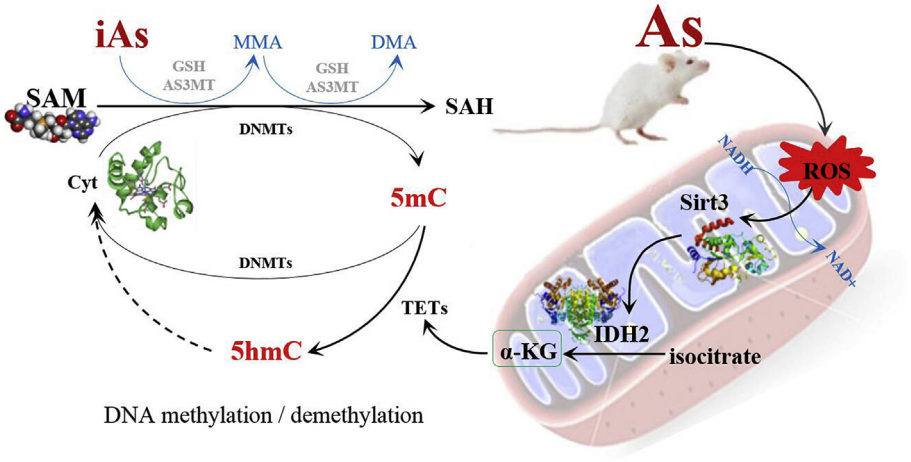

Fig. 1. Schematic of arsenic interference on DNA methylation/demethylation.