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Neurotoxicity induced by arsenic in *Gallus Gallus*: Regulation of oxidative stress and heat shock protein response

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HIGHLIGHTS

- Arsenic significantly induced neurotoxicity in chickens.
- Arsenic exposure caused oxidative stress in chicken brain tissues.
- The increased Hsps levels may play a neuroprotective function.
- Arsenic-induced neurotoxicity mechanisms are oxidative stress and Hsps response.

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ABSTRACT

Arsenic, a naturally occurring heavy metal pollutant, is one of the functioning risk factors for neurological toxicity in humans. However, little is known about the effects of arsenic on the nervous system of *Gallus Gallus*. To investigate whether arsenic induce neurotoxicity and influence the oxidative stress and heat shock proteins (Hsps) response in chickens, seventy-two 1-day-old male Hy-line chickens were treated with different doses of arsenic trioxide (As_2O_3). The histological changes, antioxidant enzyme activity, and the expressions of Hsps were detected. Results showed slightly histology changes were obvious in the brain tissues exposure to arsenic. The activities of Glutathione peroxidase (GSH-Px) and catalase (CAT) were decreased compared to the control, whereas the malondialdehyde (MDA) content was increased gradually along with increase in diet-arsenic. The mRNA levels of Hsps and protein expressions of Hsp60 and Hsp70 were up-regulated. These results suggested that sub-chronic exposure to arsenic induced neurotoxicity in chickens. Arsenic exposure disturbed the balance of oxidants and antioxidants. Increased heat shock response tried to protect chicken brain tissues from tissues damage caused by oxidative stress. The mechanisms of neurotoxicity induced by arsenic include oxidative stress and heat shock protein response in chicken brain tissues.

1. Introduction

Arsenic is a trace element widely distributed in nature and occurs as a component of over 245 minerals (Rahman et al., 2015). Naturally occurring inorganic arsenic is known to cause serious health problems (Suzuki et al., 2013). Several studies indicate that arsenic is one of the functioning risk factors for neural injury. Organisms are routinely exposure to arsenic via natural sources, industrial sources, food and drink (Mandal and Suzuki, 2002). Acute inorganic arsenic exposure has been reported to induce neuropathy and reduce motor conduction velocity with manifestations of headache, unconscious, stupefaction etc. (Grantham and Jones, 1977; Le Quesne and McLeod, 1977). Chronic exposure cause peripheral encephalopathy of sensory and motor neurons with increased vibrotactile threshold (Vahidnia et al., 2006) showing signs of numbness, loss of reflexes, and muscle weakness (Hafeman et al., 2005; Mandal and Suzuki, 2002). It has also been suggested that arsenic can activate calpain and lead the

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neurofilament light subunit degradation in rats (Florea et al., 2007).

Reports show that heavy metals are a contributing factor in the acute toxicity of peroxides in organisms (Jomova et al., 2010; Liu et al., 2013a,b). Heavy metals could cause oxidative stress by disturbing the balance of free radicals (Zhou et al., 2015). Arsenic exposure leads to the increased production of reactive oxygen species (ROS) (Liu et al., 2001) and other toxic intermediates via biotransformation of arsenic, which may subsequently cause oxidative stress and alterations of cellular system (Kojima et al., 2009). However, it is not clear how arsenic affects ROS in animal brain tissues, especially in birds. Glutathione peroxidase (GSH-Px) is regarded as the first line to protect the membrane lipids from oxidative damage (Lapointe et al., 2005). Catalase (CAT) is one of the major antioxidant enzymes and cooperates with GSH-Px to efficiently scavenge H_2O_2 to H_2O at high H_2O_2 level and does not require a reducing substrate to perform the task (Yu, 1994; Kumari et al., 2006). Both GSH-Px and CAT are considered to be significant indexes of oxidative stress induced by heavy metals and used to eliminate ROS (Nordberg and Arner, 2001). When these enzymatic scavengers cannot counteract excessive ROS, lipid peroxidation will occur (Mena et al., 2009). One of the lipid peroxidation metabolites is malondialdehyde (MDA), which arises primarily from peroxidative cleavage of polyunsaturated fatty acids in biological systems and is also used as an biomarker to balance oxidative damage (Dmitriev and Titov, 2010).

Emerging studies have proved that excessive heavy metals and free radicals in brain will induce oxidative stress and antioxidant defenses are related to the pathogenesis of neurodegenerative diseases (Halliwell, 2006). Oxidative stress induced by heavy metals can lead to a fundamental biological reaction-heat shock response (Bernstam and Nriagu, 2000). Heat shock proteins (Hsps) is regarded as a denfense system to protect organisms from different kinds of adverse conditions such as heat shock, pathogen infection, heavy metal pollution, ischemia, metabolic disorders, inflammation, stress etc. (Sreedhar and Csermely, 2004; Zhang and Zhang, 2012; Cheng et al., 2015). These inducers will probably generate abnormally folded proteins and disturb the heat shock response (Bernstam and Nriagu, 2000). Hsps can maintain key cellular processes such as protein folding, repairing and transportation (Feder and Hofmann, 1999). They are classified into Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90 according to molecular weight. Hsp27 could increase the tolerance of cell injury induced by stress (Guay et al., 1997). Hsp60 involves in the assist with the protein folding and stress protection in mitochondria (Cechetto et al., 2000). Hsp70 facilitates protein folding with the help of Hsp40 (Li et al., 2009). Hsp90 maintains the function of key proteins such as steroid receptors and protein kinases by forming specific complexes (Csermely et al., 1998). Upon exposure to environmental stimulations, these proteins are rapidly synthesized, whereas the synthesis of other proteins is decelerated (Hughes et al., 2011; Chen et al., 2012).

The toxicity effects of arsenic on the liver, immune and gastrointestinal tissues of chickens have been reported in previous studies (Guo et al., 2015a,b; Zhang et al., 2015). However, little is known about its neurological effects on birds. Therefore, we investigated the alterations in histopathology, antioxidants (GSH-Px and CAT), MDA content and Hsps (Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90) level in the brain tissues of chickens treated with arsenic trioxide (As_2O_3). Revealing the effect of arsenic on neurotoxicity in chickens, including disordered antioxidant system, increased heat shock response, and slightly tissues damage probably due to neuroprotective functions of Hsps against oxidative stress.

2. Materials and methods

2.1. Experimental animals

Seventy-two 1-day-old male Hy-line chickens were purchased from Weiwei Co. Ltd. (Harbin, China). They were maintained in the Laboratory Animal Center, College of Wildlife Resources, Northeast Forestry University, China. Over the entire experimental period, chicken were allowed ad libitum consumption of food and water.

2.2. As_2O_3 exposure

All procedures used in this study were approved by the Institutional Animal Care and Use Committee of Northeast Forestry University (Harbin, China) (UT-31; 20 June 2014). Chicken were randomly divided into four groups (18 chicken per group): the low-As group (L group), middle-As group (M group), and high-As group (H group) were fed the basal diet plus 7.5, 15, and 30 mg kg^{-1} As_2O_3, respectively. In contrast, the control group (C group) was fed the basal diet. The supplementation of As_2O_3 in the L, M, H group was according to 1/80, 1/40, and 1/20 of the median lethal dose for chicken. Six chicken of each group were selected randomly and euthanized by sodium pentobarbital after stress termination on 30, 60, and 90 d of the experiment, respectively. Then, the brain tissues (cerebrum, cerebellum, thalamus, brainstem and cerebrospinal) were excised and frozen immediately in liquid nitrogen, and stored at −80 °C until required (Liu et al., 2013a,b).

2.3. Histological observations

The brain tissues were cut into small pieces and fixed in 4% paraformaldehyde. Then, the samples were embed in paraffinwax and sectioned. Finally, the sections (5 μm) were stained with hematoxylin and eosin and observed using light microscopy (Xing et al., 2014).

2.4. Detection of the antioxidant enzymes activities and MDA content

The brain tissues taken at different time-points were homogenized in 0.9% NaCl solution and centrifuged at 3000 × g for 5 min. The supernatants were collected to determine the antioxidant function. The method used to detect the antioxidant enzyme activity and MDA level in this study was well-described in our previous study (Guo et al., 2015a,b). Brief procedures were listed as the following.

The activities of GSH-Px and CAT, and MDA content were measured in six copies using the corresponding detection kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's protocol. Absorbance of the supernatant was measured at 412, 405 and 532 nm, respectively (Guo et al., 2015a,b). The results of spectrophotometric analysis were expressed as international units in nmol per protein.

2.5. Quantitative real-time PCR of Hsp27, Hsp40, Hsp60, Hsp70, and Hsp90

Total RNA of the brain tissue samples was isolated using the Trizol reagent according to the instructions (RNAiso Plus, Takara, China) and was then treated with RNase-free DNase to remove residual DNA. Quality of the extracted total RNA was tested by a spectrophotometer (Ultrospec 1100 *pro*, Amersham Biosciences) at $OD_{260/280}$ nm. Then, the extracted total RNA (50 μg) was reverse transcribed into first-strand cDNA using the PrimeScript™ RT reagent Kit (TaKaRa, China) according to the manufacturer's