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NF-κB-mediated inflammation correlates with calcium overload under arsenic trioxide-induced myocardial damage in *Gallus gallus*

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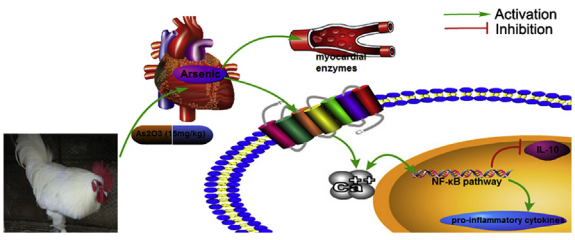
HIGHLIGHTS

- Arsenic significantly induced myocardial injury in chickens.
- Arsenic increased the activities of myocardial enzymes in chickens serum.
- Arsenic induced disbalance of calcium regulation-related genes.
- Arsenic induced Ca overload in chickens heart.
- Arsenic triggered NF-κB-dependent inflammatory response in chickens heart.

GRAPHICAL ABSTRACT



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ABSTRACT

Arsenic is a known environmental pollutant and highly hazardous toxin to human health. Due to the biological accumulation, arsenic produces a variety of cardiovascular diseases. However, the exact mechanism is still unclear. Here, our objective was to evaluate myocardial damage and determine the potential mechanism under arsenic exposure in chickens. Arsenic trioxide (As_2O_3) (1.25 mg/kg BW, corresponding 15 mg/kg feed) was administered as basal diet to male Hy-line chickens (one-day-old) for 4, 8 and 12 weeks. The results showed that As_2O_3-induced histological and ultrastructural damage in heart accompanied with significantly Ca^{2+} overload and increased the activities of myocardial enzymes. Moreover, As_2O_3 exposure significantly increased ($P < 0.05$) the mRNA levels of ITPR3, PMCA, TRPC1, TRPC3, STIM1, ORAI1 and pro-inflammatory genes, while the mRNA levels of ITPR1, ITPR2, RyR1, RyR3, SERCA, SLC8A1, CACNA1S and interleukin-10 were decreased ($P < 0.05$) by As_2O_3 exposure at 4, 8 and 12 weeks as compared with the corresponding control group. Western blot results showed that As_2O_3 exposure decreased the expression of SERCA and SLC8A1 protein, while the expression of TNF-α, NF-κB, iNOS and PMCA1 increased compared with the corresponding control group. Additionally, correlation analysis and protein-protein interaction prediction shown that NF-κB-mediated inflammatory response have a function correlation with calcium (Ca) regulation-related genes. In conclusion, this study indicated that As_2O_3-induced inflammatory response might dependent on Ca overload in myocardial damage of chickens. Our work has implications for the development of potential therapeutic approaches by resisting Ca overload for arsenic-induced myocardial damage.

1. Introduction

Arsenic is notorious for its toxic, ubiquitous, non-degradable

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and accumulative nature (Ma et al., 2017). The goal annual anthropogenic input of arsenic into the soil was estimated in between 2.84×10^7 and 9.4×10^7 kg/year (Jiang et al., 2015). Although the permissible limit for arsenic consumption in drinking water is 10 μg/L, water sources in other country like pakistan many areas are highly contaminated mention some recent study (Arain et al., 2015; Baig et al., 2016; Brahman et al., 2016). Kazi et al. (2013) have reported that arsenic residues in different tissues (leg, breast, liver and heart muscles) of chickens were 2–10 fold higher than safe limit from five poultry farms. Heart is the central organ of the circulatory system and is a primary target organ of arsenic exposure (Wang et al., 2007). Phung et al. (2017) have reported that arsenic exposure in water exceeding 10% to 50% cardiovascular risk. Long-term arsenic exposure either induces ischemic heart and cardiovasular diseases or causes various pathological responses at the molecular level of cardiomyocytes (Tseng et al., 2003). As a bio indicator species, birds have been extensively studied for environmental quality assessments in Europe and United States (Abbasi et al., 2015). Therefore, the mechanism of the effects of arsenic on bird cardiovascular health will be the subject of future studies.

Calcium ion (Ca^{2+}) has been proposed as transducer between cytosolic work and mitochondrial metabolism. Extramitochondrial Ca^{2+} can modify ATP production, via an increase in matrix Ca^{2+} content, rapidly enough to support cardiac work transitions *in vivo* (Territo et al., 2001). Changes in cytoplasmic Ca^{2+} can trigger responses as diverse as exocytosis, muscle contraction, enzyme metabolism, gene transcription and cell proliferation (Berridge et al., 2003). Mitochondiral calcium (Ca) overload is a key determinant in heart failure (Santulli et al., 2015). When the heart is overloading with Ca^{2+}, mitochondria embrace their darker side, and induce necrotic cell of the myocytes (Halestrap and Pasdois, 2009). Ca^{2+} channels are important to the biological function of muscles in the influx and efflux of it. The intracellular Ca^{2+}-release channel regulates the duration and amplitude of Ca efflux in muscle, including ryanodine receptor channel (cardiac ryanodine receptor (RyR1, RyR3)) and Ca^{2+} pump channel (SERCA) (Priori and Napolitano, 2005). Moreover, extracellular Ca^{2+}-entry channels regulates cell proliferation and muscle contraction, including: L-type voltage-dependent Ca^{2+} channel dihydropyridine receptors (CACNA1S), transient receptor potential channels (TRPC1, TRPC3 and others) (Xu and Beech, 2001). Also, Ca^{2+}-release-activated Ca^{2+} current channels (CRAC), extracellular Ca^{2+}-entry balancing channels (Na^+/Ca^{2+} exchanger (NCX (solute carrier family 8 member 1 (SLC8A1))) and plasma membrane Ca^{2+}-ATPases (PMCA1) concertedly accomplish the organelle's Ca^{2+} demand (Graier et al., 2008; Lee, 2010; Yao et al., 2016). Moreover, the diversity of Ca signals generated by inositol 1,4,5-trisphosphate receptors (IPR1, IPR2, IPR3) is thought to be largely the result of Ca (co-agonist), ATP, other adenine nucleotides and interactions with various binding proteins (Chandrasekhar et al., 2016). Arsenic can lead to peroxidation of membrane lipids, which causes membrane leakage leading to the influx of extracellular Ca (Gupta et al., 2013; Adebayo et al., 2015). Chlamydophila pneumoniae contact induces Ca^{2+} release, leading to nuclear factor-kappa B (NF-κB) pathway activation in type II cells (Wissel et al., 2005). However, the effect of arsenic exposure on Ca^{2+} signals in heart of birds still remains unclear.

It has been emphasized that a positive Ca^{2+} balance and a concomitant inflammatory state act as cofactors in the development of cardiovascular calcifications (Tetta et al., 2002). Persistent inflammation results in changes specific Ca^{2+} regulatory mechanisms, which would involve a decrease in Ca^{2+} efflux, uptake and/or binding and/or an increase in capacitative Ca^{2+} entry (Lu and Gold, 2008). Prolonged arsenic exposure could increase the expression of imflammatory molecules in human, which inducing high risk of atherosclerosis (Wu et al., 2003). NF-κB, a transcription of pro-inflammatory cytokines, triggering chronic inflammatory processes in response to circumstances including hypoxic or ischemic myocardial injury (Dhingra et al., 2010). Along with NF-κB activating, the inflammatory cytokines (cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and prostaglandin E synthase (PTGEs)) promotes myocardial infarction (Frangogiannis et al., 2002). Overexpression of TNF-α and significant increase of the activity of NF-κB play an important role in the postinfractional ventricular remodeling and progression of heart failure (Xie et al., 2005). However, there is still no report on arsenic-induced Ca^{2+} signals changes and the link between Ca^{2+} disorder and inflammation under arsenic trioxide (As_2O_3) exposure in heart of chickens.

Moreover, the chicken is increasingly used as a model species for studies of stress physiologly (Dansky and Hill, 1952; Yao et al., 2013). The aim of this study was to evaluate the arsenic toxic effects on heart of birds and to discuss the perspective of between Ca^{2+} channels and inflammation. In the present study, we choose chickens as experimental animal for monitoring ecological environment pollution caused by arsenic and then investigated myocardial enzymes contents, histological and ultrastructural changes, the expression levels of Ca^{2+} regulation-related genes and inflammatory cytokines in heart of chickens, and to assess the relationship between Ca^{2+} regulation-related genes and inflammatory cytokines, so as to provide new insights into the effect of arsenic in heart failure.

2. Materials and methods

2.1. Animals and treatment

Seventy-two 1-day-old Hy-line chickens were obtained from Weiwei Co. Ltd. (Harbin, China). They were maintained in the Laboratory Animal Center, College of Wildlife Resources, Northeast Forestry University, China (approval no. UT-31; 20 june 2014). Chickens were randomly divided into two groups (18 chickens per group), including a control group: basal diet, a As_2O_3 group: basal diet plus 15 mg/kg As_2O_3 (1.25 mg/kg BW), which represent one-eightieth the median lethal dose (LD_{50}) (50 mg/kg) for chicken, respectively. The supplement of As_2O_3 followed the method described by our previous study (Li et al., 2017). All chickens were examined for clinical signs of ill health and observed during the experiment. During the experimental period, Hy-line chickens were immunized and allowed *ad libitum* consumption of food and water (Appendix 1). All procedures and animal protocol were in accordance with the ethical standards of the institution. Six chickens in each group were selected randomly at 4, 8 and 12 weeks of the experiment and euthanized with sodium pentobarbital. The heart tissues were quickly excised and blotted, and stored at −80 °C until required for subsequent experiments.

2.2. Determination of arsenic and Ca contents in heart of chickens

The arsenic and Ca in the heart were determined using inductively coupled plasma mass spectrometry (ICP-MS) (Thermo iCAPQ, American). The instrument parameters of the equipment used are summarized in Appendix 4.

The arsenic and Ca concentrations were determined in acid digested samples according to the method of (Uluozlu et al., 2009). Briefly, heart tissues (1.000 g) of each sample were directly weighted into Teflon PTFE flasks. 10 mL of a freshly prepared mixture of concentrated HNO_3 and H_2O_2 (2:1, *v/v*) was add to each flask and was kept for 10 min at room temperature A blank digest was carried out in the same way. All sample solutions were clear.