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Regulation of autophagy factors by oxidative stress and cardiac enzymes imbalance during arsenic or/and copper induced cardiotoxicity in *Gallus gallus*☆

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ABSTRACT

Basal autophagy has an indispensable role in the functioning and maintenance of cardiac geometry under physiological conditions. Recently, increasing evidence has demonstrated that arsenic (As)/copper (Cu) play important roles in the autophagy of the heart. The current study was to evaluate whether oxidative damage by As or/and Cu was correlated with autophagy through the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway in the heart of birds. Arsenic trioxide (30 mg/kg) or/and cupric sulfate (300 mg/kg) were administered in a basal diet to male Hy-line chickens (one-day-old) for 12 weeks. The results showed that heart weight/body weight ratio decreased in the As + Cu group only at 4, 8 and 12 weeks. Moreover, we observed that As or/and Cu decreased high-density lipoprotein cholesterol (HDL-C) concentrations, increased total cholesterol (T-CHO) concentrations and cardiac enzymes activities in the serum. On the other hand, As or/and Cu significantly reduced the activities of total antioxidant (T-AOC), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px)) along with decreased nonenzymic antioxidant (glutathione (GSH)) concentrations and increased malondialdehyde (MDA) concentrations in the heart. Furthermore, As or/and Cu could induce autophagy in the heart of chickens through decreased mRNA levels of TORC1, TORC2, microtubule associated light chains 3-I (LC3-I) and increased PI3K, AKT1, Beclin1, autophagy associated gene 4B (Atg4B), microtubule associated light chains 3-II (LC3-II), autophagy associated gene 5 (Atg5) and Dynein. Meanwhile, ultrastructural examinations showed that As/Cu could result in the appearance of autolygosomes, autophagic vacuoles and double-membrane structures in the heart. In conclusion, As or/and Cu induced cardiac damage and autophagy via elevating cardiac enzymes activities, inducing oxidative stress and activating the PI3K/AKT/mTORC pathway in heart of chickens. Moreover, As and Cu had a possible synergistic relationship in the heart of chickens.

1. Introduction

Arsenic (As) and copper (Cu) are widely distributed in the natural environment and disturb the organic biological system when excessive exposure occurs. Along with the mining and screening processes for mineral resources, discharges of tailings containing As and Cu lead to long-term accumulation, which pollutes the surrounding water, land and vegetation, which in turn threatens the health of animals and humans (Hamed et al., 2017). The concentrations of As are around 12.49–169.25 mg/kg in surface sediments and 3.08–10.48 μg/L in lake water from Lake Dianchi, China (Nan et al., 2013). Moreover, it has been observed that Cu concentrations exceed 200 mg/kg in the sediments of tributaries feeding into Beung Boraphet, Thailand (Dummee et al., 2012). Because of heavy metal stability, high solvency and the great ability of biological accumulation in organisms, exposed to metals mixture has become a source of major pollution in birds and could pose a health risk to consumers. Therefore, assessing metal mixture toxicity can be useful to understand the underlying processes and mechanism of toxicity.

Long-term intake of As/Cu caused damage in the gastrointestinal, central nervous, endocrine, immune and cardiovascular systems and increased risk of hepatocellular carcinoma via disturbing mineral nutrient, oxidative stress, inflammatory response, autophagy (Ventura-Lima et al., 2011; Zhang et al., 2016). In Europe and the United States,

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birds have been extensively studied for environmental quality assessments and to test the usefulness of birds as bio-indicator species (Abbasi et al., 2015; Burger, 2013). Moreover, birds are exposed to complex mixtures of metal that are part of the complex ecosystems within which they reside. The heart is the central organ of the circulatory system. It distributes blood throughout the body by repeated, rhythmic contractions. Therefore, there is a need to understand how As or/and Cu induces myocardial damage in birds and to develop treatment methods to protect against the combined toxicity of the mixture.

It is generally accepted that As ingestion through food or water has induced various cardiac vascular diseases (CVD) including myocardial depolarization, cardiac arrhythmias and vasuclar damage (Blackfoot disease) (Khairul et al., 2017). High blood Cu concentrations have been proposed to be an independent risk factor for coronary artery disease (Kang, 2011). Persistent oxidative stress is the key to impaired homeostatic conditions, including injured sarcoplasmic reticulum and mitochondrial ultrastructure (J. Yang et al., 2017; T. Yang et al., 2017; Yao et al., 2016). Disturbing the oxidative and antioxidative balance can induce the oxidative stress and subsequently DNA damage and apoptosis. Moreover, oxidative stress may cause the cardiac damage and accompanied by increased heart weight (HW)/body weight (BW) ratio in rats (Rodriguez-Rodriguez et al., 2017). Previous evidence has shown that increased reactive oxygen species (ROS) trigger both induction of autophagy and selective targeting of damaged mitochondria by autophagosomes (Ding et al., 2010). Moreover, chromium-induced impairment of autophagic flux results from oxidative stress in rat proximal tubular cells (Liu et al., 2016).

Autophagy is a mechanism of self-cannibalization, and has been found to be implicated in the pathogenesis of cancer, neurodegeneration and heart injury (Yang et al., 2014). Terman and Brunk (2005) have indicated that autophagy is involved in the most important cardiac pathologies including myocardial hypertrophy, cardiomyopathies and ischemic heart disease. Su et al. (2017) have described that particulate matter 2.5 induces cell autophagy via the oxidative stress-mediated phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway in SD rats. Activation of the PI3K/AKT/mTORC pathway can trigger significant changes the expression of autophagy-related genes (Atgs) and induce autophagy. Beclin1 (Atg6) and class III PI3K are needed for the vesicle nucleation step of autophagy. Moreover, as a specific marker of autophagosome formation, microtubule-associated protein 1 light chain 3 (LC3; Atg8) expression by the sequential action of Atg4, Atg7 and Atg3 led to cell autophagy (Dong et al., 2016). Previous studies have found that autophagy induced by arsenite is mediated by oxidative stress, which regulates activation of the ERK1/2, PTEN and p70S6K signaling patyways in human and rats (Huang et al., 2015). Taken together, understanding oxidative stress and autophagy can provide evidence to clarify the cardiotoxicity of As or/and Cu in birds.

The mTOR pathway may respond to a variety of physiological stimuli and environmental conditions via modulation by a number of upstream signaling pathways. The study primarily focused on the expression and mechanism of autophagy in myocardial damage. To understand the autophagy in cardiomyocytes and to posses the ability to indentify potential therapeutic targets. The present study aims to assess As cardiotoxicity with the Cu based using a chicken model, so as to provide an experimental justification of oxidative damage induced by As or/and Cu was correlated with autophagy through the PI3K/AKT/mTORC pathway in the heart of birds.

2. Materials and methods

2.1. Animals model and experimental design

Animal care and all experimental procedures were performed in accordance with the Animal Welfare Guidelines of the Northeast Forestry University (approval no. UT-31; 20 June 2014) and were approved by the Institutional Animal Care and Use Committee in Harbin, China. A total of seventy-two male Hy-line chickens (1-day-old; purchased by Weiwei Co. Ltd., China) were divided into four groups, including a control group: basal diet, an As group: basal diet plus 30 mg/kg As_2O_3 (1/20 of LD_{50} (50 mg/kg)), a Cu group: basal diet plus 300 mg/kg $CuSO_4$, an As + Cu group: basal diet plus 30 mg/kg As_2O_3 and 300 mg/kg $CuSO_4$ (Yigit et al., 2012; Zhao et al., 2017). The composition of the diet and water was summarized in Supplementary files 1. The feeding and management in this study were described in Supplementary files 2. Each group of chickens was housed separately from the other to avoid the cross exposure to As_2O_3 and $CuSO_4$. Chickens were maintained in the Laboratory Animal Center, College of Wildlife Resources, Northeast Forestry University, China. All chickens were examined for clinical signs of ill health and observed during the experiment. Throughout the entire experimental period, chickens were allowed ad libitum consumption of feed and water. Six chickens in each group were selected randomly at 4, 8 and 12 weeks of the experiment and euthanized with sodium pentobarbital. HW and BW of every chickens were measured after sacrifice of the chickens at the 4, 8 and 12 weeks. The heart tissues were quickly excised and blotted. The tissues were rinsed with ice-cold 0.9% NaCl solution, frozen immediately in liquid nitrogen. The blood was collected from the heart of each chickens and it was allowed to stand overnight for clotting at 4 temperature (°C) and centrifuged at 3000 rpm for 10 min to obtain the serum. The serum and the heart tissue were stored at −80 °C for assays.

2.2. Measurement of As and Cu in the heart of chickens

The elements of As, Cu and calcium in the heart were determined using inductively coupled plasma mass spectrometry (ICP-MS) (Thermo iCAP-Q, MA, USA). The instrument parameters of the equipment used were as follows: frequency (MHz) 27.12, radio frequency power (KW) 1.55, sampling depth (mm) 6.0, plasma gas flow rate (L/min) 1.05, nebulizer pump (rpm) 40, S/C °C 2.7, oxide ions (156/140) < 2.0%, doubly charged (70/140) < 3.0% and nebulizer-type concentric. Initially, a complete digestion of the samples was performed with a microwave digestion system. Briefly, 1.0 g of each sample was diluted with a solution of 5 mL of HNO_3 (65%) and 3 mL H_2O_2 (30%) and then diluted to a final volume of 10 mL with deionized water. The samples were heated in the microwave-accelerated digestion system according to the following program: the power was ramped up for 10 min to 1800 W and held for 3 min at 100 °C, raised to 150 °C for 10 min, and held at 180 °C for 45 min. The digested samples were diluted with ultrapure water to a final volume of 50 mL and mixed well prior to ICP-MC analysis.

2.3. Assessment of serum cardiotoxicity enzymatic indices

The blood samples were collected from hearts at 4, 8 and 12 weeks. The blood samples (no hemolysis) were allowed to stand overnight for clotting at 4 °C and centrifuged at 3000 × *g* for 10 min to obtain the serum for the analysis. Serum T-CHO and HDL-C concentrations were estimated by using standard kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacture's protocol. Absorbance of the supernatant was measured at 510 nm and 546 nm. The T-CHO and HDL-C concentrations were expressed as millimole per litre of serum. Serum total cardiac enzymes (aspartate transaminase (AST), creatine kinase (CK), creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and α-hydroxybutyrate dehydrogenase (α-HBDH)) activities were estimated by using an automatic chemical analyzer (7100, Hitachi, Tokyo, Japan) according to manufacture's instruction. The serum cardiac enzymes activities were expressed as U/L.

2.4. Determination of oxidative stress indices

The heart tissues taken at different time-points were homogenized