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Effects of selenium-lead interaction on the gene expression of inflammatory factors and selenoproteins in chicken neutrophils☆

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ABSTRACT

Lead (Pb) is one of the most highly toxic metal pollutant that can cause damage to the immune system. It is known that selenium (Se) can antagonize heavy metals. To explore the toxic effects of Pb poisoning on bird immune cells, as well as the alleviating effects of Se on Pb, Se supplement and/or Pb poisoning chicken models were established. One hundred and eighty Hyline 7-day-old male chickens received either Se (1 mg Se per kg of diet), Pb (350 mg Pb per liter water) or Se + Pb in their diet and water for 90 days. Then, whole blood was collected from the four groups of chickens, and serum and neutrophils were isolated. The levels of Se and Pb in chicken serum, mRNA levels of 24 selenoproteins (GPX1, GPX2, GPX3, GPX4, Dio1, Dio2, Dio3, Txnrd1, Txnrd2, Txnrd3, SELS, SPS2, SELK, SELW1, SEP15, SEPX1, SELT, SELI, SELO, SELM, SEPN1, SEPP1, SELU, SELH) and inflammatory factors (TNF-α, COX-2, iNOS, NF-κB), and iNOS protein level in chicken neutrophils were determined, and protein-protein interaction prediction and principal component analysis were performed. The data showed that Pb exposure increased Pb content in serum, activated the NF-κB pathway, and increased the expression of selenoproteins in chicken neutrophils. Se supplements could reduce Pb concentration in serum, had a mitigative effect on the activation of the NF-κB pathway and further enhanced the upward trend of selenoprotein expression induced by Pb exposure. These results suggest that Se supplement could eliminate Pb in serum and alleviate the activation of the NF-κB pathway under Pb exposure by increasing the expression of selenoproteins.

1. Introduction

Lead (Pb) is a highly toxic heavy metal and is one of the main pollutants in terrestrial and aquatic ecosystems (Ettler et al., 2004). Pb has become a serious source of environmental pollution, and Pb-containing residues are widespread due to industrialization and urbanization. As a result of Pb pollution, residues of Pb are found not only in soil and water but also in crops in many countries (Pollution and Ores, 2011; Toan et al., 2014; Walraven et al., 2014). Heavy metals can be absorbed by organisms and bioaccumulate through every step of the food chain up to animals and even humans (Tsiridis et al., 2006). There is an increasing concern about the adverse health effects of Pb. It is already known that Pb interferes with a variety of biochemical processes and affects the normal functions of the body, including cardiovascular, hematopoietic, reproductive, gastrointestinal, renal, and nervous systems (Kosnett et al., 2007; Liu et al., 2010; Müller et al., 2008). Pb is also associated with the risk of breast cancer (Alatise and Schrauzer, 2010). Accumulating evidence indicates that the immune system is also one of the targets for Pb. Sub-chronic Pb intoxication significantly depresses macrophage functions in chickens (Kumar et al., 2000). Moreover, human chronic exposure to Pb may diminish neutrophil function (Kosnett et al., 2007).

The nuclear factor-κB (NF-κB) plays a key role in immune systems, and the inflammatory response (Bonizzi and Karin, 2004). NF-κB induces the transcription of Pro-inflammatory cytokines, chemokines, tumor necrosis factor-a (TNF-a), cyclo-oxygenase2 (COX-2) and inducible oxide synthase (iNOS). iNOS is expressed and activated by stimuli during inflammatory events and produces nitric oxide (NO), which is the key physiologic mediator in the regulation of various biological processes in addition to inducing tissue injury at the inflammatory site (Nagy et al., 2007). The sustained activation of the NF-kB by metal-activated signaling pathways can lead to chronic inflammatory processes and related diseases, including carcinogenesis (Freitas and Fernandes, 2011). It has been reported that Pb exposure causes an

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increase of inflammatory markers NO and TNF-α in brain of rats (Ghareeb et al., 2010). Wei et al. have also reported that Pb causes induction of COX-2 in a nuclear factor of activated T cells (NFAT)-dependent manner, playing a key role in mediation of inflammation reaction and oxidative stress (Wei et al., 2014). It has been reported that changes in inflammatory factors are associated with neutrophil survival and the recruitment and infiltration abilities of these cells (Cross et al., 2008; Shah and Walsh, 2007; Speyer et al., 2003).

Selenium (Se) is one of the essential micronutrients, and it interacts with heavy metals in vivo as a part of the natural metal detoxification processes. Previous studies found that Se can protect liver and kidneys of rats from the impairments induced by Pb exposure (Flora et al., 1983; Othman and El, 1998). Xu et al. also found that Se and/or Pb could change the ion profiles in livers of chickens, suggesting that Se may protect against the toxic effects of Pb (Tong et al., 2015). Se plays an important role in inflammation and immunity, is involved in initiating immunity, and regulates excessive immune responses and chronic inflammation (Huang et al., 2011). Se primarily elicits its function via selenoproteins in vivo, which contain a unique amino acid selenocysteine (Sec) in its active center. It has been reported that selenoproteins are expressed in a variety of tissues in chicken, including muscle, liver, kidney, heart, spleen, and lung (Jiang et al., 2015). In addition, selenoproteins are expressed in sheep neutrophils (Hugejiletu et al., 2013). Zhao et al. reported that the gene expression of selenoproteins was significantly decreased and that immune function was suppressed in immune tissue of the thymus in chickens fed a Se-deficient diet (Zhao et al., 2015). Zhang et al. reported that the gene expression of a proportion of selenoproteins is up-regulated in supplemented diets with above-adequate levels of Se (Zhang et al., 2014). Moreover, Hugejiletu et al. reported that supranutritional Se-yeast supplementation or Se inadequacy may stimulate innate immune responses and change the expression of selenoproteins in sheep neutrophils (Hugejiletu et al., 2013). Boyne and Arthur (1986) noted a decrease in the ability of neutrophils to kill phagocytosed Candida albicansin in cows suffering from Se deficiency (Boyne and Arthur, 1986). Furthermore, suppression of selenoprotein W (SEPW1/SELW) in cultured chicken splenic lymphocytes resulted in an increase in mRNA expression of inflammation factors greater than levels seen in response to H2O2-induced oxidative stress.

Numerous studies have reported that Pb exposure triggers various tissues damage in organisms, and Se has an antagonistic effect on this damage. Neutrophils are important phagocytic cells in peripheral blood, and studies have shown that Pb could reduce the function of neutrophils (Kosnett et al., 2007). Excessive expression of inflammatory factors can cause tissue damage (Kanuri et al., 2011; Sehnert et al., 2013), but the effect of Pb on the expression of inflammatory factors in peripheral blood neutrophils is not clear. Se has antagonistic effects on Pb toxicity and Se primarily elicits its function via selenoproteins in vivo. Therefore, we established chicken models of sub-chronic Pb poisoning and Se antagonism and observed a clear effect of Pb on inflammatory factor expression in chicken peripheral blood neutrophils and the relationship of selenoprotein expression and inflammatory factor expression, thus providing evidence to clarify the immune toxicity of Pb and the antagonistic role of Se.

2. Materials and methods

2.1. Animals model and experimental design

Hyline male chickens (1 day old; Weiwei Co. Ltd., Harbin, China) were fed with basal diet (containing 0.49 mg/kg Se) and conventional drinking-water for 1 week. Then, they were randomly divided into four groups: Con, Se, Pb, and Se + Pb. Control group was fed basal diet (0.49 mg Se per kg diet) and conventional drinking water (Pb-free in water); Se group were fed Na_2SeO_3 (analytical reagent grade, Tianjin, China)-added basal diet at the level of 1 mg Se per kg diet (super-nutritional Se but not Se toxicity) and conventional drinking water; Pb group were fed basal diet and $(CH3OO)_2Pb$ (analytical reagent grade, Tianjin, China)-added drinking water at the level of 350 mg Pb per Liter water, according to median lethal dose (LD_{50}) of Pb acetate for cocks (Vengris and Mar, 1974) and the need of the chicken experiment in toxicology (Klaassen and Watkins, 2013); Se + Pb group were fed Na_2SeO_3-added basal diet at the level of 1 mg Se per kg diet and $(CH3OO)_2Pb$-added drinking water at the level of 350 mg Pb per Liter water. Throughout the entire experimental period, chickens were allowed ad libitum consumption of food and water. The chickens were maintained in the Laboratory Animal Center, College of Veterinary Medicine, Northeast Agricultural University, China. Whole blood was quickly taken at 90 days old, then serum were isolated for determining Se and Pb levels, and neutrophils were separated using kits according to the manufacturer's instructions (TBD, China) and stored at −80 °C for isolating RNA and protein. In this study, neutrophils from 15 chickens were collected per group at the 5 sampling events, and 5 neutrophil simples per group were used in the official test (n = 5), which was repeated thrice. All of the procedures used in this study were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University.

2.2. Detection of serum Se and Pb concentrations

Se and Pb in chicken serum were examined by inductively coupled plasma mass spectrometry (ICP-MS; Thermo iCAPQ, USA). The parameters of the equipment used were as follows: frequency (MHz) 27.12, reflect power (KW) 1.55, sampling depth (mm) 5.0, torch-H (mm) 0.01, torch-V (mm) −0.39, carrier gas (L/min) 1.05, nebulizer pump (rpm) 40, S/C temperature (°C) 2.7, oxide ions (156/140) < 2.0%, doubly charged (70/140) < 3.0%, and nebulizer-type concentric.

Se and Pb concentrations were determined in the acid digest of samples according to the method of Uluozlu et al. (Uluozlu et al., 2009). 1 mL of serum was digested with 2 mL H2O2(30%) and 5 m L HNO3 (65%) and then diluted to a final volume of 10 m L with deionized water. All sample solutions were clear. Samples were digested in a microwave system applied at 3 min for 1800 W at 100 °C, 10 min for 1800 W at 150 °C, and 45 min for 1800 W at 180 °C. Deionized water is used to be blank, was carried out at the same time in the same manner. All digested samples were filled with ultrapure water to the 50 mL final volume and mixed well prior to analysis by ICP-MS.

2.3. Primer design

Primer Premier Software (PREMIER Biosoft International, USA) was used to design specific primers for glutathione peroxidase 1 (GPX1), glutathione peroxidase 2 (GPX2), glutathione peroxidase 3 (GPX3), glutathione peroxidase 4 (GPX4), iodothyronine deiodinase 1 (Dio1), iodothyronine deiodinase 2 (Dio2), iodothyronine deiodinase 3 (Dio3), thioredoxin reductase 1 (Txnrd1), thioredoxin reductase 2 (Txnrd2), thioredoxin reductase 3 (Txnrd3), selenoprotein S (SELENOS/VIMP/SELS), selenophosphate synthetase 2 (SPS2), selenoprotein K (SELENOK/SELK), SEPW1, selenoprotein F (SELENOF/SEP15), methionine sulfoxide reductase B1 (MSRB1, also known as SEPX1), selenoprotein T (SELENOT/SELT), selenoprotein I (SELENOI/SELI), selenoprotein O (SELENOO/SELO), selenoprotein M (SELENOM/SELM), selenoprotein N (SELENON/SEPN1), selenoprotein P-Z (SELENOPZ/SEPP1), selenoprotein U (SELU), selenoprotein H (SELENOH/C11orf31/SELH), inflammation factors (NF-κB, TNF-α, COX-2, and iNOS), and β-actin based on known chicken sequences (Table 1).

2.4. Total RNA isolation and reverse transcription

Total RNA was isolated from the cell samples using TRIzol reagent according to the manufacturer's instructions (Takara, China). The dried RNA pellets were resuspended in 120 μL of diethyl-pyrocarbonate-

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