Selenium Alleviated Oxidative Stress-Mediated Complex Poisoning Mechanism in Lead-Treated Chicken Kidneys: Inflammation, Heat Shock Response, and Autophagy

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Abstract

Lead (Pb) is a toxic environmental contaminant, and exerts renal toxicity. It is known that selenium (Se) performs antagonistic effect on Pb poisoning. However, biological events during the process were not well understood in chicken kidneys. The purpose of this research was to investigate mitigative mechanism of Se on Pb poisoning from point of view of oxidative stress, inflammation, heat shock response, and autophagy in chicken kidneys. One hundred and eighty male Hyline chickens (7-day-old) were randomly divided into the control group (offering standard diet and potable water), the Se group (offering Na₂SeO₃-added standard diet and potable water), the Pb group (offering standard diet and (CH₃OO)₂Pb-added potable water), and the Pb+Se group (offering Na₂SeO₃-added standard diet and (CH₃OO)₂Pb-added potable water). On 30th, 60th, and 90th days, kidneys were removed to perform the studies of histological structure, oxidative stress indicators, cytokines, heat shock proteins, and autophagy in the chicken kidneys. The experimental results indicated that Pb poisoning changed renal histological structure; decreased catalase, glutathione-s-transferase, and total antioxidative capacity activities; increased hydrogen peroxide content; induced mRNA and protein expression of heat shock proteins; inhibited interleukin (IL)-2 mRNA expression, and induced IL-4 and IL-12 β mRNA expression; inhibited mammalian target of rapamycin mRNA and protein expression, and induced autophagy-related gene mRNA and protein expression in the chicken kidneys. Supplement of Se mitigated the above changes caused by Pb. In conclusion, Pb induced oxidative stress, inflammation, heat shock response, and autophagy and Se administration alleviated Pb poisoning through mitigating oxidative stress in the chicken kidneys.

Introduction

Lead (Pb) is a ubiquitous toxic environmental contaminant. Although laws and regulations has been implement to control Pb pollution, it is still an important health issue in many countries (Mohammadi et al., 2014). Continuous Pb exposure to human beings and animals constitutes various health risk, and even lead to death (Patrick 2006). Previous study has reported that the children exposure to Pb born between 1972 and 1973 in Dunedin and New Zealand may affect mental health in adulthood (Sancar 2019). Exposure to low level Pb was associated with chronic kidney disease has been studied in adults of United Kingdom (Green & Pain 2019). Pb pollution led to millions of bird death annually around the world (De Francisco et al., 2003) and even drove California condors (*Gymnogyps californianus*) to the edge of extinction from 1997 to 2010 (Finkelstein et al., 2012).

Oxidative stress caused by prooxidant/antioxidant imbalance with reactive oxygen species (ROS) overproduction plays a crucial role in renal injury, as it has been considered a central aggravating factor (Thomas et al., 2019). There are cross-talk between oxidative stress and inflammation in preeclampsia (Tenorio et al., 2019). Heat shock proteins (HSPs) are a family of proteins produced by cells in response to exposure to stressful conditions and are primary mitigators of cell stress (Hang et al., 2018). Autophagy occurs at basal levels to preserve cellular homeostasis by recycling proteins and organelles which can also act in response to oxidative stress (Roca-Agujetas et al., 2019). Oxidative stress,

inflammation, heat shock response, and autophagy have been described in many studies as prominent factors in mediating many pathological alterations in response to toxic agents (Ayoub et al., 2017). Pb induced oxidative stress which led to autophagy in the spleens of chickens (Han et al., 2017) and mice (Corsetti et al., 2017). Autophagy was intertwined with inflammation, and cytokines can help mediate this interaction (Ge et al., 2018). Autophagy was decreased in nude mice with hepatocellular carcinoma and was inversely correlated with HSPs expression (Chen et al., 2019). However, it is not well characterized whether there is an interplay between these factors or any combination of them in mediating harmful mechanisms of pathological alterations in Pb-treated kidneys.

Selenium (Se) is a necessary trace element for organisms (Sun et al., 2018). As an antioxidant, Se plays critical roles in maintaining intracellular redox balance. Our previous study found that Se could alleviate Pb-caused oxidative stress (Huang et al., 2018), heat shock response (Huang et al., 2017), and inflammatory damage (Wang et al., 2017) in chicken testes. In addition, recent studies reported that Se-yeast inhibited the initiation of autophagy and enhanced autophagic clearance in the brains of Alzheimer's disease mice (Song et al., 2018). Although antagonistic effect of Se on Pb was investigated, underlying molecular mechanism remained to be elucidated. Therefore, in current study, we designed interaction model of Pb and Se in chickens and detected histological alterations, oxidative stress indexes, mRNA and protein expression of interleukins, HSPs, and autophagy-related genes to reveal antagonistic mechanism of Se on Pb in the chicken kidneys.

Material And Methods

Animal model

Hyline chickens (1-day-old) were provided standard diet (containing 0.49 mg/kg Se) (D) and potable water (Wang et al., 2017) during 7 days acclimatization. Then, 180 healthy birds were randomly divided into four groups with 45 numbers: the control group, the Se group, the Pb group, and the Pb+Se group, respectively. the control group was given D and W; the Se group received a diet enriched with Na₂SeO₃-added D (containing 1 mg/kg Se) (SeD) and W; the Se group was offered (CH₃OO)₂Pb through drinking water (containing 350 mg/L Pb) (PbW), following median lethal dose of Pb acetate for cocks and the need of chicken experiment in toxicology (Vengris & Mare 1974); and the Pb+Se group supplied with SeD and PbW. Na₂SeO₃ and (CH₃OO)₂Pb were analytical reagent grade and were purchased from Tianjinzhijuan Chemical Reagen Co., Ltd. Tianjin, China. According to feeding standard, the chickens were provided food and water *ad libitum* at a temperature of 22 ± 2°C under 12 h-light/12 h-dark cycles in Laboratory Animal Center, Animal Medical College, Northeast Agricultural University (Harbin, China) until the end of experiment.

Tissue samples

On 30th, 60th, and 90th days of the experiment, respectively, 15 birds with 12 h fasting from each group were euthanized. Then the kidneys were immediately separated and cleaned with ice-cold saline. The first part of the sample was immediately frozen in liquid nitrogen and stored at -80°C to detect mRNA and

protein expression. The second part of the sample was homogenized to determine oxidative stress indexes. The third part of the sample was fixed in 4% paraformaldehyde solution and stayed at least 24 h to perform microstructure observation. The last part of the sample was fixed in 2.5% glutaraldehyde phosphate buffer saline to observe ultrastructure.

Microstructure

Preparation of sections described in our present papers was processed as previously described (Huang et al., 2019b). Briefly, the kidney tissues fixed with paraformaldehyde solution were dehydrated in gradient alcohol (30, 50, 70, 90, 100, and 100%), were embedded in paraffin, and were sectioned to nominal thicknesses of 4 μm . The sections were stained with hematoxylin and eosin. Finally, the sections were subjected to microscopic examination (Eclipse 80i, Nikon, Tokyo, Japan) and photographs were taken.

Ultrastructure

The samples were cut into blocks with the size of $1.0 \times 1.0 \times 1.0$ mm and were immediately fixed in 2.5% glutaraldehyde phosphate buffer saline at 4°C for 3 h (pH 7.2). The blocks were rinsed in 0.1 mol/L PBS, put in 1% osmium tetroxide at 4°C for 1 h, and were rinsed in 0.1 mol/L PBS again. The tissues were impregnated and were embedded with epoxy resins. The obtained sections were counterstained with uranyl acetate and lead citrate after ultrathin section. The ultrastructure of chicken kidneys was observed and was photographed using transmission electron microscope (Model JEM-1200EX, Jeol Jem, Japan).

Oxidative stress indexes

Kidney tissues were pulverized in a homogenization buffer (0.32 M $(\text{NH}_4)_2\text{SO}_4$, 0.10 mM EDTA, 25% glycerol, and 0.05 M Tris-HCl pH 7.9). Obtained sample was homogenized on ice using a homogenizer. The homogenate solution was centrifuged at 16,000 g and 4°C for 5 min. Obtained supernatant was used for determining T-AOC, GST, and CAT activities and H_2O_2 content in chicken kidneys using kits produced by Nanjing Jiancheng Bioengineering Institute (Nanjing, China) following the manufacturer's instructions. All samples were detected in duplicate in a single assay to avoid interassay variation.

Relative mRNA expression analysis

Primer sequence and Genbank accession numbers of detected genes were listed in Table 1. GAPDH served as internal reference gene. The special primers were synthesized by Invitrogen Biotechnology Co. Ltd. in Shanghai, China.

Table 1
Special primers of genes used for real-time PCR

Gene name	Accession number	Primer sequence	Size of the products (bp)
IL-2	AY510091	Forward 5'-GAACCTCAAGAGTCTTACGGGTCTA-3'	111
		Reverse 5'-ACAAAGTTGGTCAGTTCATGGAGA-3'	
IL-4	AJ621249	Forward 5'-GTGCCCACGCTGTGCTTAC-3'	82
		Reverse 5'-AGGAAACCTCTCCCTGGATGTC-3'	
IL-12 β	AJ564202	Forward 5'-TGTCTCACCTGCTATTTGCCTTAC-3'	87
		Reverse 5'-CATACACATTCTCTCTAAGTTTCCACTGT-3'	
HSP27	NM_205290.1	Forward 5'-ACACGAGGAGAAACAGGATGAG-3'	158
		Reverse 5'-ACTGGATGGCTGGCTTGG-3'	
HSP40	NM_001199325.1	Forward 5'-GGGCATTCAACAGCATAGA-3'	151
		Reverse 5'-TTCACATCCCCAAGTTTAGG-3'	
HSP60	NM_001012916.1	Forward 5'-AGCCAAAGGGCAGAAATG-3'	208
		Reverse 5'-TACAGCAACAACCTGAAGA-3'	
HSP70	NM_001006685.1	Forward 5'-CGGGCAAGTTTGACCTAA-3'	250
		Reverse 5'-TTGGCTCCCACCCTATCTCT-3'	
HSP90	NM_001109785.1	Forward 5'-TCCTGTCCTGGCTTTAGTTT-3'	143
		Reverse 5'-AGGTGGCATCTCCTCGGT-3'	
ATG5	gi 449273598	Forward 5'-GATGAAATAACTGAAAGGGAAGC-3'	208
		Reverse 5'-TGAAGATCAAAGAGCAAACCAA-3'	
Beclin 1	NM_001006332.1	Forward 5'-CAGACACGCTGCTGGACC-3'	114
		Reverse 5'-TCTCCTTGTCATCCTCGTTCA-3'	
Dynein	NM_001006519.1	Forward 5'-TGGGATAATCGCAGCAATAAGA-3'	243
		Reverse 5'-AGGGAAGGACATGCAAGTAACAG-3'	
LC3-I	gi 311294088	Forward 5'-GCATCCAAACAAAATCCCAGT -3'	246
		Reverse 5'-AAGCCATCCTCATCCTTCTCCT-3'	
LC3-II	NM_001031461.1	Forward 5'-CTTCTTCCTCCTGGTGAACG-3'	131
		Reverse 5'-GCACTCCGAAAGTCTCCTGA-3'	

Gene name	Accession number	Primer sequence	Size of the products (bp)
mTOR	XM_417614.5	Forward 5'-GGACTCTTCCCTGCTGGCTAA-3'	143
		Reverse 5'-TACGGGTGCCCTGGTTCTG-3'	
GAPDH	NM_204305.1	Forward 5'-AGAACATCATCCCAGCGT-3'	128
		Reverse 5'-AGCCTTCACTACCCTCTTG-3'	

Total RNA was extracted from kidney tissues with TRIzol reagent following the method provided by the manufacturer (Invitrogen, China). Spectrophotometer (Healthcare Bio-Sciences AB, Sweden) was used to determine RNA purity. OD260/OD280 was between 1.8 and 2.1, and met the experimental requirements. Complementary DNA (cDNA) was synthesized with PrimeScript™ RT reagent Kit (TaKaRa, Japan) in a volume of 60 µL (containing 5 µg of the total RNA) according to the manufacturer's instructions. Obtained cDNA was diluted fivefold with sterile water and was kept at -20°C until next step.

Quantitative real-time PCR was performed using LightCycler® 96 (Roche, Life Science) with the SYBR® PrimeScript™ RT-PCR Kit (Roche, Switzerland) following manufacturer's instructions. The reaction system comprised 0.3 µL of forward primer (10 µM), 0.3 µL of reverse primer (10 µM), 1 µL of diluted cDNA, 3.4 µL of sterile distilled water, and 5 µL of 2 × SYBR green PCR master mix (Takara, China). The PCR procedure was as follows: at 52°C for 2 min and 95°C for 10 min; followed by 40 cycles of amplification and quantification at 95°C for 15 s, at 60°C for 60 s, and at 95°C for 15 s; and at 60°C for 20 s. Each sample was repeated three times. Relative mRNA expression was calculated according to the $2^{-\Delta\Delta CT}$ method.

Western blot analysis

Kidney tissues (about 50 mg) were cut from each kidney and washed in saline, and then were sliced and homogenized in sodium dodecyl sulfate (SDS) lysate. Homogenate solution was centrifuged and were extracted supernatant. Protein quantification was detected with BCA protein assay kits (Thermo Scientific, USA). Then, proteins were put into SDS-PAGE gel and were transferred to the membranes of nitrocellulose at 200 mA for 1 h. The membranes were put into 5% skim milk to block at 4°C for 12 h. The antibodies were diluted to 1:1000 (HSP27), 1:1000 (HSP40), 1:1000 (HSP60), 1:500 (HSP70), 1:500 (HSP90), 1:100 (LC3-I and LC3-II), 1:500 (Dynein and mTOR), and 1:1000 (ATG5 and Beclin 1), respectively. After being washed for four 5-min periods with PBST, the membranes reacted with secondary antibodies against rabbit IgG (1:1000, Santa Cruz, USA) at 37°C for 1 h. Then the membranes were washed for four 5-min periods. Western blotting detection kits (Thermo Scientific, USA) were used for detecting protein expression. The membranes were exposed X-ray films. Then, protein levels were analyzed using image VCD gel imaging system (Beijing Sage Creation Science and Technology Co. Ltd., Beijing, China). The GAPDH signal was used as an internal reference.

Statistical analysis

All experiment data were presented as the mean $\pm$ standard deviation (SD). One-way and two-way analyses of variance (ANOVA) were performed using SPSS (version 21.0, SPSS Inc., Chicago, IL, USA). Kruskal-Wallis ANOVA test and Mann-Whitney *U* test were used to compare difference among multiple groups. Statistical significance was assigned at $P < 0.05$.

Results

Histology aerations

To explore the effect of Pb on chicken kidneys and mitigative effect of Se on Pb poisoning, chickens were treated with Pb and Se for 90 days. Histology alterations of chicken kidneys were shown in Figure 1 on 90th day. In the control group (Figure 1(A1)) and the Se group (Figure 1(B1)), glomerular structure was clear and glomerular cavity was clearly visible. In the Pb group (Figure 1(C1)), glomerulus was swollen, the boundaries of renal cyst were unclear, renal tubular epithelial cells were swollen, inflammatory cells infiltrated extensively, and vascularization occurred compared with the control group. In the Pb+Se group (Figure 1(D1)), glomerulus was slightly swollen, the margin of renal cyst was little blurred, renal tubular epithelial cells were slightly swollen, and inflammatory infiltration decreased compared with the Pb group.

Ultrastructure results of chicken kidneys were as follows: Organelles were normal in the control group (Figure (A2)) and the Se group (Figure (B2)). In the Pb group (Figure (C2)), the nucleus was hyperchromatic and autophagosomes were visible after Pb treatment for 90 days. In the Pb+Se group (Figure (D2)), the number of autophagosome was less than that in the Pb group.

T-AOC, GST, and CAT activities and H₂O₂ content in chicken kidneys

To assess the effect of Pb treatment on oxidative stress and mitigative effect of Se, oxidative stress indexes including T-AOC, GST, CAT, and H₂O₂ were measured on 30th, 60th, and 90th days. As shown in Figure 3, there were significant differences ($P < 0.05$) in T-AOC (Figure 3 (A)), GST (Figure 3 (B)), and CAT (Figure 3 (C)) activities and H₂O₂ (Figure 3 (D)) content among different groups except there were no significant difference ($P > 0.05$) in T-AOC, GST, and CAT activities and H₂O₂ content between the control group and the Se group at three time points. T-AOC, GST, and CAT activities was lowest in the Pb group, followed by in the Pb+Se group, in the Se group and the control group at three time points. Change tendency of H₂O₂ content was opposite to that of T-AOC, GST, and CAT activities at three time points. In addition, T-AOC, GST, and CAT activities decreased significantly ($P < 0.05$) with the increase of treatment duration, but H₂O₂ content showed opposite trend ($P < 0.05$) in the Pb group.

Relative mRNA expression of IL-2, IL-4, and IL-12 β

To investigate the effects of Pb and Se on the inflammation of chicken kidneys, the expression of IL-2, IL-4, and IL-12 β was detected on 30th, 60th, and 90th days (Figure 3). Pb treatment caused a notable decrease in IL-2 mRNA expression and increase in IL-4 and IL-12 β mRNA expression in chicken kidneys ($P < 0.05$). Se administration significantly induced IL-2 mRNA expression and reduced IL-4 and IL-12 β mRNA expression ($P < 0.05$). In addition, IL-2 mRNA expression decreased significantly ($P < 0.05$) and IL-4 and IL-12 β mRNA expression increased significantly ($P < 0.05$) with the increase of treatment duration in the Pb group.

Relative mRNA and protein expression of HSP27, HSP40, HSP60, HSP70, and HSP90

To detect the effect of Pb and Se on heat shock response in chicken kidneys, mRNA and protein expression of HSP27, HSP40, HSP60, HSP70, and HSP90 was measured on 30th, 60th, and 90th days (Figure 4). Pb treatment led to notably increase ($P < 0.05$) in mRNA and protein expression of HSP27, HSP40, HSP60, HSP70, and HSP90 in the chicken kidneys, while chickens with Se administration showed significant recovery ($P < 0.05$) of mRNA and protein expression of HSPs as compared with the Pb group (Figure 3). However, there was no significant difference ($P > 0.05$) in mRNA and protein expression of HSP27, HSP40, HSP60, HSP70, and HSP90 between the control group and the Se group. In addition, mRNA expression of all the above detected HSPs increased significantly ($P < 0.05$) with the increase of treatment duration in the Pb group.

Relative mRNA and protein levels of ATG5, Beclin 1, Dynein, LC3-I, LC3-II, and mTOR

To determine mitigative effect of Se on autophagy in Pb-treated chicken kidneys, the expression of autophagy-related genes including ATG5, Beclin 1, Dynein, LC3-I, LC3-II, and mTOR was evaluated on 90th day. As shown in Figure 5, a notable increase in mRNA and protein expression of ATG5, Beclin 1, Dynein, LC3-I, and LC3-II and decrease in mRNA and protein expression of mTOR was observed in the Pb-treated chicken kidneys ($P < 0.05$, vs. the control group or the Se group). However, Se intervention significantly decreased the expression of above autophagy-related genes except that mTOR was increased ($P < 0.05$).

Discussion

Frequent environmental and occupational exposure to Pb has been well-established to induce organ toxicity and subsequent adverse pathological consequences. Many of these diverse toxic effects are manifested at both cellular and molecular levels and share common mechanisms of action across various tissues and organs. Excessive Pb exposure can cause histological alterations of chicken kidneys and renal damage of rats (Jin et al., 2017; Wang et al., 2013). Pb treatment caused tubular degeneration, cell swelling, and inflammatory infiltration in rat kidneys (Yurekli et al., 2009). In this study, we found that

Pb exerted toxicity in chicken kidneys according to typical features of pathological alterations after Pb treatment, such as swollen glomeruli, inflammatory infiltration, and vascularization.

It is well known that oxidative stress is a core mechanism of Pb toxicity due to imbalance in oxidant/antioxidant homeostasis (Chander et al., 2014). Excessive Pb was absorbed to tissues which overproduced H_2O_2 (Lopes et al., 2016). H_2O_2 , a typical oxidant, is capable of diffusing throughout the mitochondria and across cell membranes and producing many types of cellular injury (Ray & Husain 2002). Moreover, Pb depletes cells antioxidants, particularly thiolcontaining compounds and antioxidant enzymes (CAT and T-AOC) during oxidative stress (Lopes et al., 2016). GST is one of the predominant antioxidant enzymes against oxidative stress in living organisms (Hayes et al., 2005). CAT is a primary defense against oxidative stress, and can catalyze the conversion of H_2O_2 into oxygen and water (Ahn et al., 2005). At the same time, it is a potential target of Pb (Flora et al., 2008). T-AOC is used to measure the amount of free radical purge and evaluate antioxidant status (Zhang et al., 2012b). It has been reported that changes in GST, T-AOC, CAT, and H_2O_2 were associated with Pb poisoning in rat kidneys (Apaydin et al., 2016; Oyagbemi et al., 2015). Pb can decrease T-AOC, GST, and CAT activities; and increase H_2O_2 content; and cause oxidative stress in the bursa of Fabricius of chickens (Jiao et al., 2017). In our findings, the damage was clearly demonstrated by the production of H_2O_2 , which was accompanied by depletion in the activities of antioxidant enzymes (T-AOC, GST, and CAT) in the chicken kidneys upon exposure to Pb compared to non-treated group, suggesting that Pb caused renal injury and oxidative stress in the chicken kidneys. These results supported the fact that Pb toxicity induced renal injury by increasing oxidative stress, and similar phenomena had been reported previously (Gargouri et al., 2019; Kelainy et al., 2019). The consequence of the decrease in the level of T-AOC, CAT and GST was due to direct binding of Pb with their sulfhydryl groups (Saxena et al., 2005), altering their function or suppressing their activities by Pb (Ercal et al., 2001). In addition, the decrease in CAT activity was also attributed to scavenging of H_2O_2 in Pb-intoxicated chickens. Therefore, the alterations of oxidative status, either by the overproduction of oxidants or deficit in antioxidant activity, was one of direct consequences of Pb toxicity in the chicken kidneys. In addition, we also found that T-AOC, CAT, GST, and H_2O_2 changed in a time-dependent effect in the Pb-induced chicken kidneys. It suggested that oxidative stress was gradually strengthened with Pb treatment duration. Our previous experiment also reported that Pb had a time-dependent effect on T-AOC, GST, and CAT activities and H_2O_2 content in chicken bursa of Fabricius (Jiao et al., 2017).

Inflammatory response is the first line of defense in response to all forms of cellular injuries and clears cellular damage and initiates cellular repair (Sochocka et al., 2017). But when inflammatory response is inappropriate it can lead to damage of surrounding normal cells. One of the events that occurred following oxidative stress is inflammatory response. It has been reported that increased oxidative stress might stimulate the expression of cytokines leading to increased inflammation (Reuter et al., 2010). IL-4 and IL-12 β were proinflammatory mediators and IL-2 was anti-inflammatory one. Thus, in present study, IL-2, IL-4, and IL-12 β were selected for mRNA expression analysis. We found that Pb treatment increased IL-4 and IL-12 β and decreased IL-2 in the chicken kidneys, suggesting that Pb enhanced inflammatory

process after oxidative stress in the chicken kidneys. The process of abnormal Pb invasion-caused oxidative stress triggered inflammatory response, through the cytokine production, such as IL-4 and IL-12 β , which led to a reduction in the anti-inflammatory cytokine production, such as IL-2, and consequently, cells were damaged. In fact, inflammatory damage has been known to occur during the process of inflammation after Pb treatment, which was clearly seen from our histological results. Moreover, the increase of H₂O₂ level could cause structural damage to membranes. Our findings suggested a crosstalk between Pb-induced oxidative stress and inflammation. Other researchers also concluded that there was a relationship between oxidative stress and inflammation. Previous study has found that lipopolysaccharide decreased CAT activity and increased H₂O₂ content with the increase of IL-4 in chicken myocardials (Liu et al., 2020). Also, H₂O₂ content increased, GST and CAT activities decreased, and oxidative stress occurred which prompt expression of IL-4 in aspirin-treated mouse stomachs (Abd El-Ghffar et al., 2018). In addition, we also found that IL-2, IL-4, and IL-12 β mRNA expressed in a time-dependent effect in the Pb-induced chicken kidneys, which suggested that inflammatory response was gradually strengthened with Pb treatment duration.

Oxidative stress is also responsible for activation of heat shock response (Kalmar & Greensmith 2009). HSPs also play a role in sensing oxidative stress, are involved in restoring physiological protein conformation during and after oxidative stress, and which are characteristic features of a number of pathological conditions. In response to oxidative stress, the expression of HSPs elevates dramatically which is notable as a pervasive adaptation mechanism in organisms that enables them to survive and adapt to different environmental stressors (Kalmar & Greensmith 2009). Increased levels of HSPs were an indicative of initiation of a stress response for mediating cellular protection and indicated tissue damage (De Maio 2011). Heat shock response can protect against toxicity caused by excess heavy metals (Zhu et al., 2014). Previous studies reported that Pb increased HSPs (HSP27, HSP40, HSP60, HSP70, and HSP90) mRNA expression in peripheral blood neutrophils and hearts of chickens (Huang et al., 2019a; Xing et al., 2018). In the present study, we observed high expression of HSPs (HSP27, HSP40, HSP60, HSP70, and HSP90) mRNA and protein caused by Pb exposure in the chicken kidneys, reflected the activation of this intracellular buffer system, which responds to oxidative stress when the antioxidant enzyme (T-AOC, GST, and CAT) exhaustion occurs (Kalmar & Greensmith 2009). The findings of this study indicated that Pb exposure resulted in the activation of HSPs under burden of oxidative stress. Interestingly, increasing evidence suggests that there is a complementary regulation between HSPs and inflammation (Kalmar & Greensmith 2009). Besides, inflammation is itself a stimulus for upregulation of HSPs production (Humphries et al., 2005). Therefore, in our study, elevated HSPs, on the one hand, antagonized the mentioned Pb-induced oxidative stress, on the other hand, inhibited inflammation. In addition, we also found that HSP27, HSP40, HSP60, HSP70, and HSP90 mRNA expression increased in a time-dependent effect in the Pb-induced chicken kidneys. It suggested that HSP response was gradually strengthened with Pb treatment duration.

Autophagy is an intracellular lysosomal degradation process, which plays an important role in regulating normal cell homeostasis, and is considered as one of cellular defense against increased oxidative stress

(Zhang et al., 2017). Autophagy contributed to Pb-induced nephrotoxicity has been elucidated in primary rat proximal tubular cells (Song et al., 2017). Pb promoted protein levels of Beclin1, c and LC3-II; and induced autophagy in rat hippocampi (Zhang et al., 2012a). Pb increased mRNA and protein levels of ATG5, Beclin-1, Dynein, LC3-I, and LC3-II; decreased mRNA and protein levels of mTOR; and induced autophagy in chicken spleens (Han et al., 2017). Our present research is consistent with above studies. We found that Pb treatment promoted mRNA and protein expression of Beclin 1, Dynein, ATG 5, LC3-I, and LC3-II; and inhibited mRNA and protein expression of mTOR. Furthermore, we found typical features of autophagy, formation of autophagosome, through the ultrastructure of chicken kidneys. Molecular and histology evidence of our study demonstrated that Pb induced autophagy in the chicken kidneys. Therefore, we concluded that elevated HSPs (HSP27, HSP40, HSP60, HSP70, and HSP90) were also a trigger for autophagy in Pb treatment group.

Previous studies have confirmed potent antioxidative and anti-inflammatory activities of Se. Some researches demonstrated that Se mitigated Pb-induced oxidative stress by means of increasing T-AOC, GST, and CAT activities; and decreasing H₂O₂ content in *Cyprinus carpio* livers (Ozkan-Yilmaz et al., 2014), chicken bursa of Fabricius (Jiao et al., 2017), and chicken splenic lymphocytes (Zhao & Zhang 2018). Previous study have reported that Se can mitigate increase of IL-4 and IL-12 β mRNA expression, and the decrease of IL-2 mRNA expression in Pb-treated chicken bursa of Fabricius (Jiao et al., 2017). Also, Se alleviated the increase of IL-4 and IL-12 β mRNA expression and the decrease of IL-2 mRNA expression caused by Pb has illuminated in chicken neutrophils (Xing et al., 2018). In addition, Se can mitigate Pb-caused increase of HSPs and autophagy. Pb poisoning induced mRNA expression of HSP27, HSP40, HSP60, HSP70, and HSP90; and Se administration alleviated the above HSPs changes in chicken testes (Wang et al., 2017). Se exhibited significant antagonistic roles against Pb-induced increases of HSP (27, 40, 60, 70, and 90) mRNA expression in peripheral blood neutrophils and hearts of chickens (Huang et al., 2019a; Xing et al., 2018). Se was reported by one of the articles to alleviate spleen toxicity in a chicken model induced by Pb via the modulation of oxidative stress, inflammation, and autophagy (Han et al., 2017). Se alleviated protein increase of ATG5, Beclin1, Dynein, LC3-I, and LC3-II and protein decrease of mTOR in Cd-induced chicken pancreas (Liu et al., 2018). In our study, all alterations caused by Pb were ameliorated by treatment with Se. Such effect was attributed to kidney tissue antioxidant capacity because of better antioxidant supply, thus reducing the oxidative damage represented by the reduction of T-AOC, GST, and CAT and the rise of H₂O₂. The ability of Se to neutralize oxidative stress could be due to facilitating chelation with Pb in the chicken kidney tissues, resulting in reduced Pb accumulation in the body through its potential antioxidant efficacy (Li et al., 2005). Se alleviated oxidative stress, which naturally alleviated these downstream events. Therefore, Se alleviates heat shock response and autophagy.

Conclusion

Excessive Pb led to oxidative stress, which further triggered a defensive response including heat shock response, inflammatory response, and autophagy in the chicken kidneys. Se alleviated heat shock

response, inflammatory response, and autophagy in the Pb-treated chicken kidneys. In addition, the effects of Pb poisoning had time-dependent manners in the chicken kidneys.

Declarations

Acknowledgments

All authors have read the manuscript and agreed to submit it in its current form for consideration for publication in Environmental Science and Pollution Research. This paper has not been published or accepted for publication. It is not under consideration at another journal.

Ethics approval and consent to participate

All procedures used in this experiment were approved by the Northeast Agricultural University's Institutional Animal Care and Use Committee under protocol number SRM-06. All chickens in the research were housed in the Laboratory Animal Center, College of Veterinary Medicine, Northeast Agricultural University. Feed and drinking water were offered ad libitum during the research period.

Consent to Publish

Not applicable.

Authors' contributions

XT conceived and designed the study. ZM and WY performed the literature search, generated the figures and table, and wrote the manuscript. ZM, WY, YW collected and analyzed the data, and critically reviewed the manuscript. YT and XT supervised the study and reviewed the manuscript. All authors submitted comments on drafts and read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

Figure 1

Effects of Pb, Se, and their co-treatment on histological alterations of chicken kidneys on 90th day. G: glomerulus; RTEC: renal tubular epithelial cell; II: inflammatory infiltration; V: vacuole; N: nucleus; A: autophagosome. Paraffin sections of kidney tissues from the control group (A1), the Se group (B1), the Pb group (C1) and the Pb+Se group (D1) were stained with hematoxylin-eosin (400 ×). The Pb group showed glomerular fibrosis, blurring boundary of renal cysts, vacuolization, the swollen of renal tubular epithelial cells, and inflammatory infiltration (C1). The pathological injuries in the Pb+Se group were alleviated by Se administration (D1). Ultrathin sections of kidney tissues from the control group (A2), the Se group (B2), the Pb group (C2) and the Pb+Se group (D2) were tinted with uranyl acetate and lead citrate (12, 000 ×). The Pb group showed mitochondrial swelling and mitochondrial cristae fracture (C2). The histological alterations in the Pb+Se group were alleviated by Se administration (D2).

Figure 2

Effects of Pb, Se, and their co-treatment on oxidative stress indicators in chicken kidneys on 30th, 60th, and 90th days. T-AOC (A), GST (B), and CAT (C) activities and H₂O₂ (D) content were determined with commercial assay kits. Bars represent mean $\pm$ SD (n = 5/group). In the same time point, the bars sharing different lowercase letters represent statistically significant differences between the groups (P < 0.05); the bars with a common letter are not significant different (P > 0.05). In the same group, the bars sharing different uppercase letters represent statistically significant differences in the different time points (P < 0.05); the bars with a common letter are not significant different (P > 0.05).

Figure 3

Effects of Pb, Se, and their co-treatment on mRNA expression of cytokines in chicken kidneys on 30th, 60th, and 90th days. IL-2 (A), IL-4 (B), and IL-12 β (C) mRNA expression were determined by real-time PCR. Bars represent mean $\pm$ SD (n = 5/group). In the same time point, the bars sharing different lowercase letters represent statistically significant differences between the groups (P < 0.05); the bars with a common letter are not significant different (P > 0.05). In the same group, the bars sharing different uppercase letters represent statistically significant differences in the different time points (P < 0.05); the bars with a common letter are not significant different (P > 0.05).

Figure 4

Effects of Pb, Se, and their co-treatment on mRNA and protein expressions of HSPs in chicken kidneys. HSP27 (A), HSP40 (B), HSP60 (C), HSP70 (D), and HSP90 (E) mRNA expressions were determined by real-time PCR and their protein expressions (F) were determined using Western-blot. Bars represent mean $\pm$ SD (n = 5/group). In the same time point, the bars sharing different lowercase letters represent statistically significant differences between the groups ($P < 0.05$); the bars with a common letter are not significantly different ($P > 0.05$). In the same group, the bars sharing different uppercase letters represent statistically significant differences in the different time points ($P < 0.05$); the bars with a common letter are not significant different ($P > 0.05$).

Figure 5

Effects of Pb, Se, and their co-treatment on mRNA and protein expression of autophagy-related genes in chicken kidneys on the 90th day. ATG5, Beclin 1, Dynein, LC3-I, LC3-II, and mTOR mRNA expression (A) were determined by real-time PCR and their protein expression (B) were determined using Western-blot. Bars represent mean $\pm$ SD (n = 5/group). Bars with different lowercase letters were significant different in different groups ($P < 0.05$).

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