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Selenium Protects against Lead-induced Apoptosis via Endoplasmic Reticulum Stress in Chicken Kidneys

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Abstract Lead (Pb) is a toxic heavy metal and can harm organisms by inducing apoptosis. Selenium (Se), an essential trace element for humans and animals, can alleviate heavy metal toxicity. The aim of our study is to investigate alleviative effect of Se on Pb-induced apoptosis via endoplasmic reticulum (ER) stress in chicken kidneys. One hundred and eighty male chickens were randomly divided into four groups at 7 days of age and were fed with commercial diet (containing 0.49 mg/kg Se) and drinking water, Na_2SeO_3-added commercial diet (containing 1 mg/kg Se) and drinking water, the commercial diet and $(CH_3OO)_2Pb$-added drinking water (containing 350 mg/L Pb), and Na_2SeO_3-added commercial diet (containing 1 mg/kg Se) and $(CH_3OO)_2Pb$-added drinking water (containing 350 mg/L Pb), respectively. On the 30th, 60th, and 90th days of the experiment period, 15 chickens in each group were euthanized and the kidneys were collected. Following contents were performed: kidney ultrastructure; nitric oxide (NO) content; inducible nitric oxide synthase (iNOS) activity; relative messenger RNA (mRNA) and protein expression of iNOS, ER-related genes (glucose-regulated protein (GRP)78, GRP94, activating transcription factor (ATF)4, ATF6, and iron-responsive element (IRE)), and apoptosis-related genes (caspase-3 and B cell lymphoma-2 (Bcl-2)); and caspase-12 protein expression. The results indicated that Pb changed kidney ultrastructural structure; decreased Bcl-2 mRNA and protein expression; and increased NO content, iNOS activity, relative mRNA and protein expression of iNOS, ER-related genes, and caspase-3 and caspase-12 protein expression. Se attenuated above changes caused by Pb. Pb had time-dependent manners on NO content, GRP78, GRP94, ATF4, IRE, and caspase-3 mRNA expression. Se attenuated Pb-induced apoptosis via ER stress in the chicken kidneys.

Keywords Lead · Selenium · Chicken kidney · Apoptosis · Endoplasmic reticulum stress · NO

Introduction

Lead (Pb) could cause water and soil pollution, and then affected the health of humans and animals, even affected biodiversity in wild birds through Pb accumulation in food chains. In the Ludhiana, Jalandhar, and Malerkotla areas of India, industrial activities caused Pb pollution in waters and soils and Pb accumulation in cabbages, cauliflowers, and Indian mustard [1]. In some provinces of China, such as Yunnan, Guizhou, and Sichuan, Pb pollution in waters, soils, vegetables, and crops caused by mining activities led to high blood Pb level, malacosteon, and kidney damage in local children [2]. In Pyeongtaek, Korea, the cumulation of inorganic fertilizer and pesticide led to Pb accumulation in gray herons, intermediate egrets, and black-crowned night herons, and seven of the birds even died [3]. Pb poisoning was an important cause of death in endangered white-headed ducks (*Oxyura leucocephala*) and swans (*Cygnus* sp.) [4]. The kidney is a target organ of Pb toxicity. Exposure to Pb led to damage in the kidneys of rats [5] and chickens [6]. Some researchers found that Pb-induced apoptosis was a probable mechanism

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of Pb toxicity. Pb exposure induced apoptosis in chicken erythrocytes [7] and rat proximal tubular cells [8, 9].

A large amount of nitric oxide (NO) can cause apoptosis [10]. Inducible nitric oxide synthase (iNOS) can synthesize NO. In some cell types, endoplasmic reticulum (ER) stress pathway is involved in NO-induced apoptosis [10]. Glucose-regulated protein (GRP)78, GRP94, activating transcription factor (ATF)4, ATF6, and iron-responsive element (IRE) are involved in ER stress [11]. caspase-12 is an apoptosis gene and is located on the ER. The activation of caspase-12 could cause the activation of caspase-3, and then cause apoptosis [12]. NO long-lasting production can promote apoptosis by altering Bcl-2 family proteins and caspase family proteases [13]. Excess Pb upregulated NO content and iNOS activity in chicken livers [14]. Mingwei Xing found that arsenic (As) induced iNOS messenger RNA (mRNA) and protein expression in chicken gastrointestinal tracts [15]. Excess Pb can increase NO content in rat kidneys [5], GRP78 protein expression in rat astrocytes [16], caspase-3 activity in rat kidneys [5], and caspase-3 protein expression in rat livers [17]; decrease Bcl-2 protein expression in the kidneys [5] and livers [17] of rats; and cause apoptosis in the kidneys [5] and livers [17] of rats.

Selenium (Se) is an essential trace element for animals [18] and is involved in various biological processes [19, 20]. Se can antagonize toxicity induced by heavy metals, such as mercury in K-562 cells [21] and in residents [22], cadmium (Cd) in chicken livers [23], and Pb in chicken bursa of Fabricius [24]. Se alleviated Pb-induced iNOS mRNA and protein expression in chicken neutrophils [25], Cd-induced ER stress in chicken kidneys [26] and neutrophils [27], and apoptosis in chicken livers [23] and neutrophils [27]. However, alleviative effect of Se on Pb-induced apoptosis via ER stress in chicken kidneys has not yet been completely understood. Therefore, we designed the experiment to investigate alleviative effect of Se on Pb-induced apoptosis via ER stress in chicken kidneys.

Materials and Methods

Animal Model

One hundred and eighty 1-day-old Hyline male chickens were fed a commercial diet with 0.49 mg/kg Se and drinking water for 7 days. The chickens were randomly divided into four groups: the control group, the Se group, the Pb group, and the Se/Pb group. The chickens in the control group were fed a commercial diet and drinking water. The chickens in the Se group were fed $Na_2SeO_3 \cdot 5H_2O$ (analytical reagent grade, Tianjin, Zhiyuan Chemical Reagent Co. Ltd., Tianjin, China) plus a commercial diet containing 1 mg/kg Se and drinking water. The chickens in the Pb group were fed a commercial diet and $(CH_3OO)_2Pb \cdot 3H_2O$ (analytical reagent grade, Tianjin, China) plus drinking water contaminated with 350 mg/L Pb. The use of Pb dose was conducted in the needs of experiment toxicology [28], according to a median lethal does (LD50) of Pb for chickens [29]. The chickens in the Se/Pb group were fed $Na_2SeO_3 \cdot 5H_2O$ plus a commercial diet containing 1 mg/kg Se and $(CH_3OO)_2Pb \cdot 3H_2O$ plus drinking water contaminated with 350 mg/L Pb. The chickens were maintained in the Laboratory Animal Center, College of Veterinary Medicine, Northeast Agricultural University (Harbin, China). Food and water were provided ad libitum for chickens during the entire experimental period. All procedures used in this experiment were approved by the Northeast Agricultural University's Institutional Animal Care and Use Committee under the approved protocol number SRM-06.

Tissue Sample Collection

Fifteen chickens were randomly selected from four groups, and then euthanized on the 30th, 60th, and 90th days of the experiment, respectively. The kidneys were immediately excised and washed with ice-cold 0.9% NaCl solution. Every kidney tissue was divided into three parts. One part was fixed with 2.5% glutaraldehyde phosphate-buffered saline (*v*/*v*, pH 7.2) for ultrastructure observation. One part was homogenized to detect NO content and iNOS activity. The last part was frozen in liquid nitrogen and stored in a −80 °C refrigerator for quantitative real-time polymerase chain reaction (PCR) and Western blot.

Ultrastructure Observation

On the 90th day of the experiment, the kidney tissues from four groups were cut into small blocks (1.0 mm × 1.0 mm × 1.0 mm). These blocks were stained using 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (*v*/*v*, pH 7.2) at 4 °C for 3 h, and 1% osmium tetroxide (*v*/*v*) at 4 °C for 1 h. Then the tissues were dehydrated in graded series of ethanol (50, 70, 90, and 100% ethanol for 10 min, respectively). The samples were embedded with epoxy resins and cut into ultrathin sections. The ultrathin sections were impregnated in magnesium-uranyl acetate and Pb citrate. Kidney ultrastructure was observed under a transmission electron microscope.

NO Content and iNOS Activity

NO content and iNOS activity were measured using NO and iNOS detection kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Relative mRNA Expression of iNOS, GRP78, GRP94, ATF4, ATF6, IRE, Bcl-2, and caspase-3

Primer Sequences

The specific primers of iNOS, GRP78, GRP94, ATF4, IRE, ATF6, Bcl-2, caspase-3, and β-actin published in GenBank are shown in Table 1. β-actin was used as an internal reference gene. The primers were synthesized by Invitrogen Biotechnology Co. Ltd. (Shanghai, China).

Total RNA Extraction and Reverse Transcription

Total RNA was extracted from kidney tissues using RNAiso Plus reagent according to the manufacturer's instructions (Takara, Japan). The concentration and purity of the total RNA were determined using GeneQuant 1300 spectrophotometer (Healthcare Bio-Sciences AB, Sweden) at 260/280 nm. The total RNA was immediately used to synthesize complementary DNA (cDNA). Reverse transcription (RT) reaction system (60 μL) (Haigene, China) contained 6 μL of total RNA, 1.5 μL of RNase inhibitor (40 U/μL), 3 μL of dNTP mixture (10 mM each), 6 μL of 10 × RT buffer, 3 μL of golden MLV reverse transcriptase, 3 μL of 20 × oligo dT (25) and random primer, and 37.5 μL of RNase-free H_2O. The reaction conditions were at 30 °C for 15 min, at 55 °C for 50 min, and at 80 °C for 10 min. The synthesized cDNA was diluted five times with sterile water and then was stored in a −20 °C refrigerator for real-time quantitative PCR.

Real-time Quantitative PCR

Real-time quantitative PCR was performed using LightCycler®96 Real-Time PCR System according to the manufacturer's instructions (Roche Life Science, Shanghai, China). The reaction system (10 μL) consisted of 1 μL of cDNA, 3.4 μL of sterile distilled water, 5 μL of 2 × SYBR green PCR master mix (Takara, China), 0.3 μL of forward primer (10 μM), and 0.3 μL of reverse primer (10 μM). The PCR reaction program was at 95 °C for 10 min, degeneration at 95 °C for 2 min, 40 cycles at 95 °C for 15 s, and at 60 °C for 1 min. Every sample was measured three times. The melting curve analysis showed only one peak for each PCR product. Relative mRNA abundance was calculated according to the method of Pfaffl [30].

Western Blot Analysis

The proteins of iNOS, GRP78, GRP94, ATF4, ATF6, IRE, caspase-12, Bcl-2, and caspase-3 were extracted on the 90th day in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. SDS-polyacrylamide gel (containing H_2O, 30% acrylamide mix, Tris-Hcl buffer (pH 8.8), 20% SDS, 20% 2-aminophenol 4-sulfonic acid, and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine) was prepared according to molecular size of proteins. The concentration of 30% acrylamide mix in SDS-polyacrylamide gel was 10% for iNOS; 12% for caspase-12, Bcl-2, and caspase-3; and 15% for GRP78, GRP94, ATF4, ATF6, and IRE, respectively. Proteins were separated and transferred onto nitrocellulose membranes in a tank transfer apparatus

Table 1 Gene special primers used in the experiment

Gene	Serial number	Primer sequence	Primer length	Product length
iNOS	NM_204961	Forward 5′-CCTGGAGGTCCTGGAAGAGT-3′ Reverse 5′-CCTGGGTTTCAGAAGTGGC-3′	20 19	82
GRP78	NM_205491.1	Forward 5′-GAATCGGCTAACACCAGAGGA-3′ Reverse 5′-CGCATAGCTCTCCAGCTCATT-3′	21 21	118
GRP94	NM_204289.1	Forward 5′-CAAAGACATGCTGAGGCGAGT-3′ Reverse 5′-TCCACCTTTGCATCCAGGTCA-3′	21 21	186
IRE	NM_001285501.1	Forward 5′-CTACAGGTCGCTCCTCACATC-3′ Reverse 5′-ATCAGTCCTTCTGCTCCCATCT-3′	21 21	101
ATF4	AB013138.1	Forward 5′-TCACCCAATGACAACCCG-3′ Reverse 5′-TCACCTTTGCTGACGCTACC-3′	18 20	100
ATF6	XM_422208.5	Forward 5′-CGTCGTCTGAACCACTTACTGA-3′ Reverse 5′-CCTTCTTTCCTAACAGCCACAC-3′	22 22	101
Bcl-2	NM_205339	Forward 5′-ATCGTCGCCTTCTTCGAGTT-3′ Reverse 5′-ATCCCATCCTCCGTTGTCCT-3′	20 20	150
Caspase-3	NM_204725.1	Forward 5′-CATCTGCATCCGTGCCTGA-3′ Reverse 5′-CTCTCGGCTGTGGTGGTGAA-3′	19 20	104
β-actin	L08165.1	Forward 5′-CCGCTCTATGAAGGCTACGC-3′ Reverse 5′-CTCTCGGCTGTGGTGGTGAA-3′	20 20	93

(containing Tris-glycine buffer and 20% methanol) at 100 mA for 1 h. The membranes were blocked with 5% nonfat milk at 4 °C overnight. The membranes were incubated at 37 °C for 2 h with the diluted primary antibodies iNOS (1:500), GRP78 (1:500), GRP94 (1:500), ATF4 (1:600), ATF6 (1:600), IRE (1:600), caspase-12 (1:200), Bcl-2 (1:500), and caspase-3 (1:100), respectively. After being washed for a third time (15 min each time) with phosphate-buffered saline (containing 10 mM Tris-HCl (pH 7.6), 100 mM NaCl, and 0.1% Tween 20), the membranes were incubated at 37 °C for 1 h with peroxidase-conjugated secondary antibodies against rabbit IgG (1:1000, Santa Cruz, USA) and were washed thrice (15 min each time) with the phosphate-buffered saline again. The signal was detected using X-ray films (TransGen Biotech Co., Beijing, China). The optical density was measured using Image VCD gel imaging system (Beijing Sage Creation Science And Technology Co. Ltd., Beijing, China).

Statistical Analysis

Statistical analysis was performed using SPSS for Windows (version 17.0; SPSS Inc., Chicago, IL, USA) in two-way ANOVA for the kit data and mRNA expression, and in one-way ANOVA for the protein expression data. All results were expressed as the mean ± standard deviation. The comparisons of results were verified by nonparametric Kruskal-Wallis ANOVA and Mann-Whitney *U* tests. Different uppercase letters were significantly different ($P < 0.05$) among different time points in the same group. Different lowercase letters were significantly different ($P < 0.05$) among different groups at the same time point.

Results

Ultrastructure Observation

Kidney histological study was used in our experiment as shown in Fig. 1. The cells in the control (Fig. 1(a)) and Se (Fig. 1(b)) groups showed a clear cell nucleus (N), a homogeneous cytoplasm, and an intact mitochondria (MI). The cells in the Pb group showed shrinkage of cytoplasm and chromatin (Fig. 1(c)), small cell size (Fig. 1(c)), nuclear chromatin agglutination (Fig. 1(d)), and ER amplification (Fig. 1(d)). The cells in the Se/Pb group showed densely stained nuclei, partial margination of chromatin (Fig. 1(e)), and ER amplification (Fig. 1(e and f)).

NO Content, iNOS Activity, and iNOS mRNA and Protein Expression

NO content (Fig. 2 (a)), iNOS activity (Fig. 2 (b1)), and iNOS mRNA expression (Fig. 2 (b2)) for 30, 60, and 90 days and iNOS protein expression (Fig. 2 (b3)) for 90 days in chicken kidneys from four groups are shown in Fig. 2. There were no significant differences ($P > 0.05$) of NO content, iNOS activity, and iNOS mRNA expression at all three time points and iNOS protein expression between the control group and the Se group. Compared with the control, Se, and Se/Pb groups, all above indexes increased significantly ($P < 0.05$) in the Pb group. Compared with the control and Se groups, all above indexes increased significantly ($P < 0.05$) in the Se/Pb group except iNOS mRNA expression for 30 days. NO content and iNOS mRNA expression increased significantly ($P < 0.05$) with the increase of time in the Pb group. iNOS activity for

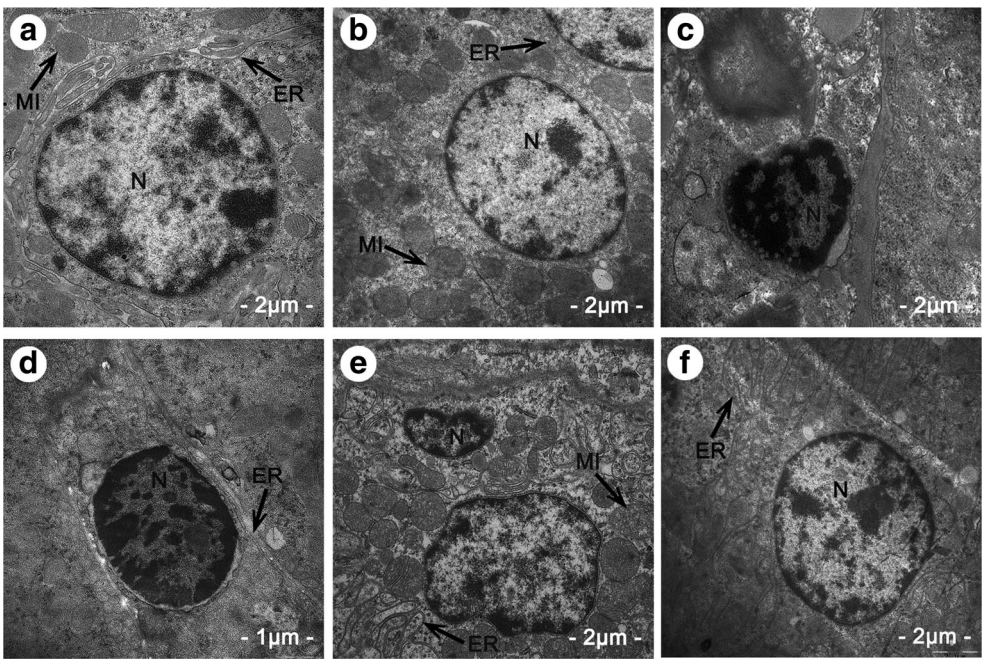

Fig. 1 Cell ultrastructure. **a** The control group (× 15,000). **b** The Se group (× 15,000). **c** The Pb group (× 15,000). **d** The Pb group (× 20,000). **e**, **f** The Se/Pb group (× 15,000). *N* nucleus, *MI* mitochondria, *ER* endoplasmic reticulum

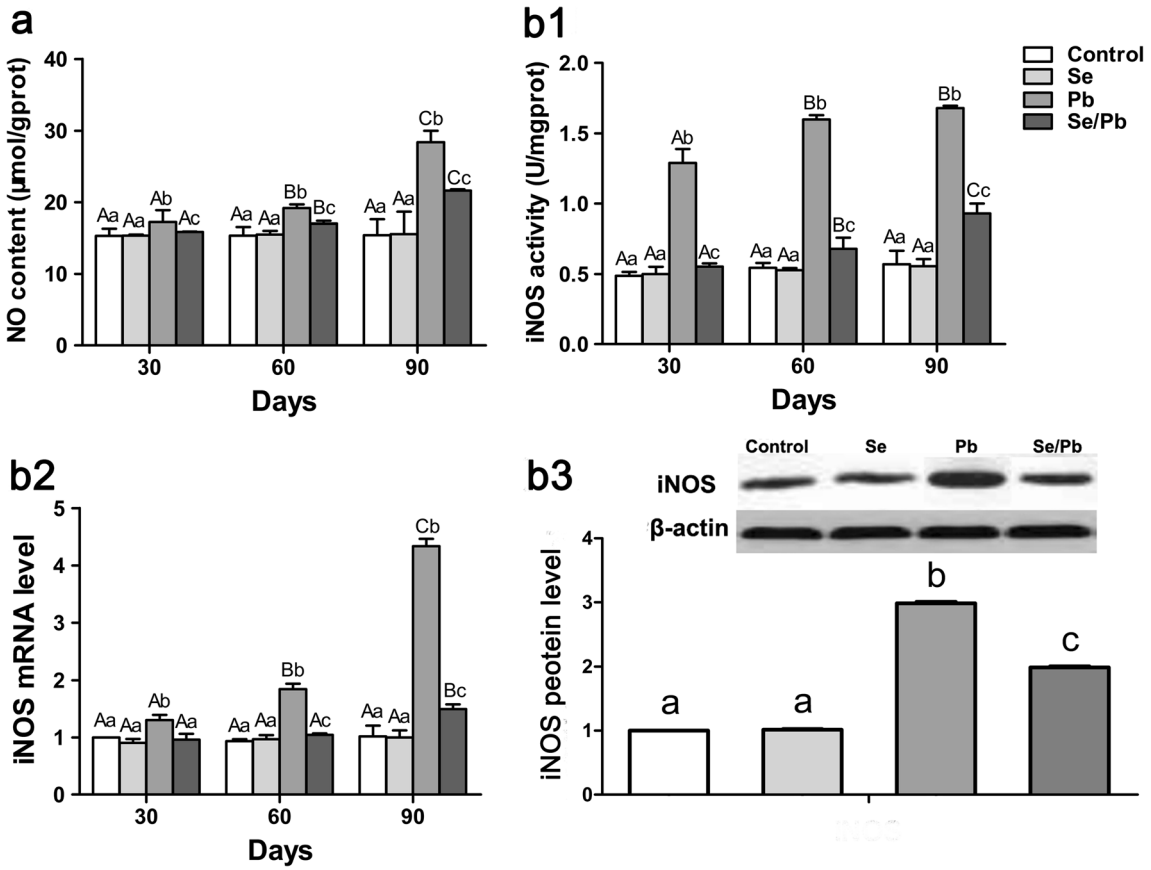

Fig. 2 *a–b3* NO content, iNOS activity, and iNOS mRNA and protein expression

60 and 90 days was significantly higher ($P < 0.05$) than that for 30 days in the Pb group.

Relative mRNA and Protein Expression of ER-related Genes

We detected relative mRNA expression for 30, 60, and 90 days and protein expression for 90 days of ER-related genes (GRP78 (Fig. 3 (a1 and a2)), GRP94 (Fig. 3 (b1 and b2)), ATF4 (Fig. 3 (c1 and c2)), ATF6 (Fig. 3 (d1 and d2)), and IRE (Fig. 3 (e1 and e2))) to investigate alleviative effect of Se on Pb-induced apoptosis via ER stress in chicken kidneys. Our results showed that there were no significant differences ($P > 0.05$) of all above genes on all the time points between in the control group and in the Se group. Relative mRNA and protein expression of the five ER-related genes in the Pb group was significantly higher ($P < 0.05$) than that in the control, Se, and Se/Pb groups except GRP78 mRNA expression for 30 days and IRE protein expression in the Se/Pb group. Relative mRNA and protein expression of the five ER-related genes in the Se/Pb group was significantly higher ($P < 0.05$) than that in the control and Se groups except mRNA expression of ATF6 for 60 days and IRE for 30 days, and protein expression of GRP94 and ATF4. Relative mRNA expression of GRP78, GRP94, ATF4, and IRE increased significantly ($P < 0.05$) with the increase of time in the Pb group. ATF6 mRNA expression for 90 days was significantly higher ($P < 0.05$) than that for 30 and 60 days in the Pb group.

Relative mRNA and Protein Expression of Apoptosis-related Genes

As shown in Fig. 4, protein expression of caspase-12 (Fig. 4 (a)) and mRNA and protein expression of Bcl-2 (Fig. 4 (b1 and b2)) and caspase-3 (Fig. 4 (c1 and c2)) were detected in chicken kidneys. We found that there were no significant differences ($P > 0.05$) of mRNA and protein expression of all above genes between the control group and the Se group. caspase-12 protein expression and caspase-3 mRNA and protein expression in the Pb group were significantly higher ($P < 0.05$) than those in the control, Se, and Se/Pb groups. caspase-12 protein expression and caspase-3 mRNA and protein expression in the Se/Pb group were significantly higher ($P < 0.05$) than those in the control and Se groups. In contrast, Bcl-2 mRNA and protein expression in the Pb group was significantly lower ($P < 0.05$) than that in the control, Se, and Se/Pb groups. Bcl-2 mRNA and protein expression in the Se/Pb group was significantly lower ($P < 0.05$) than that

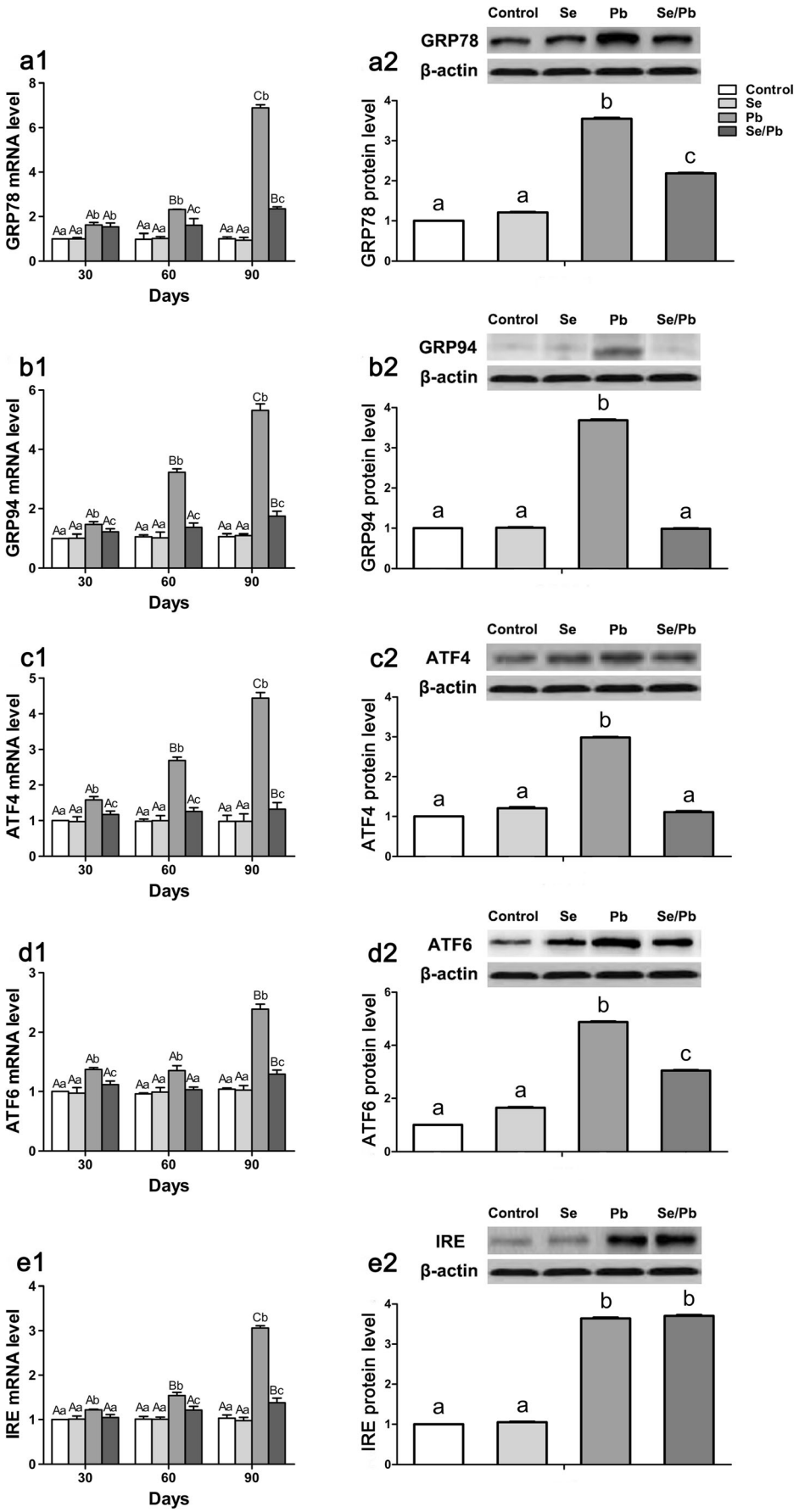

Fig. 3 *a1–e2* Relative mRNA and protein expression of ER-related genes

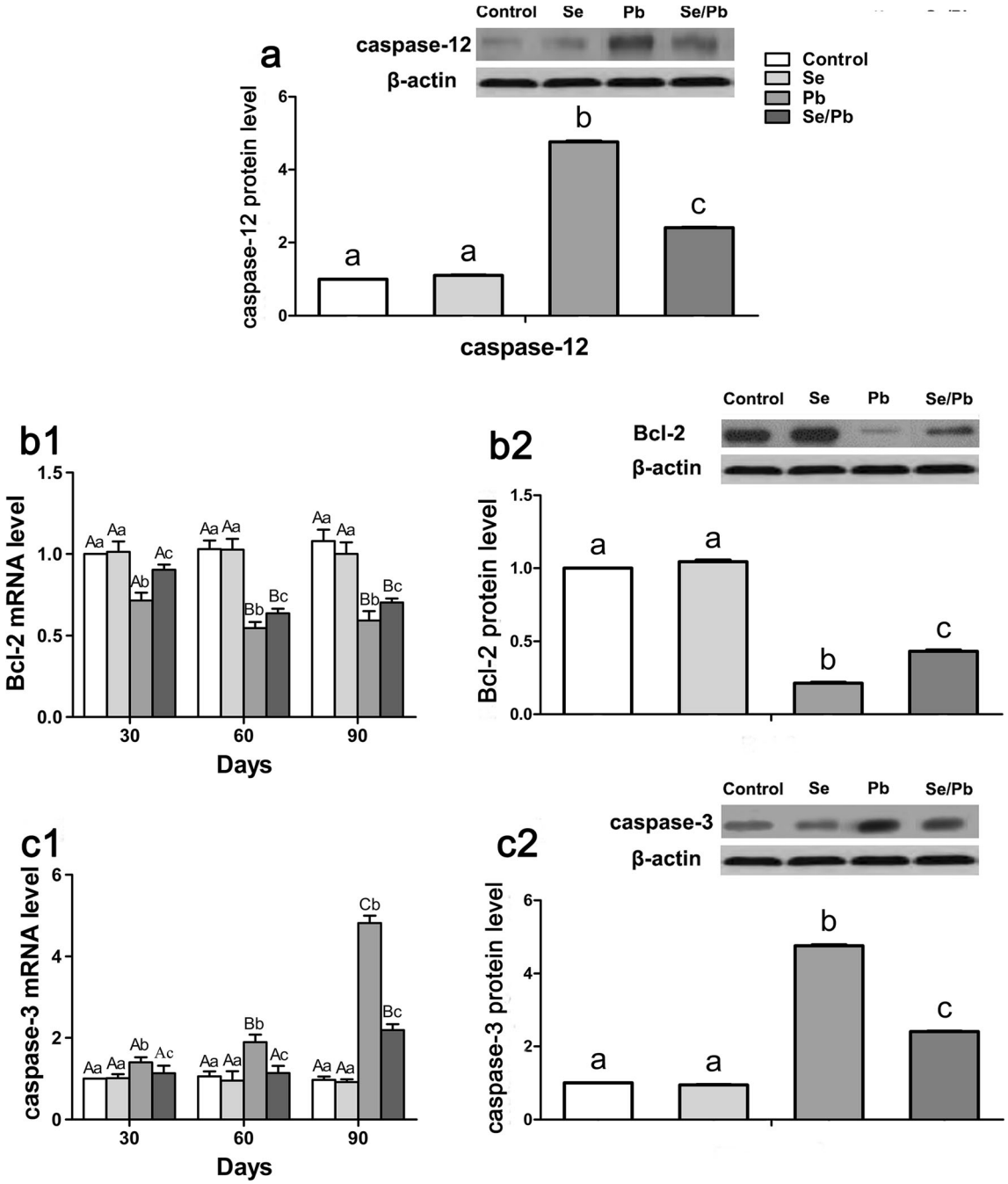

Fig. 4 *a–c2* Relative mRNA and protein expression of apoptosis-related genes

in the control and Se groups. caspase-3 mRNA expression increased significantly ($P < 0.05$) with the increase of time in the Pb group. Bcl-2 mRNA expression for 60 and 90 days was significantly lower ($P < 0.05$) than that for 30 days in the Pb group.

Discussion

Pb pollution led to Pb poisoning in wild birds. Rafael Mateo and Ronda de Toledo [4] found that 17 species of birds of prey suffered Pb poisoning in Europe, and some of them were near threatened, such as the white-tailed eagle (*Haliaeetus albicilla*), or endangered, such as the Spanish imperial eagle (*Aquila adalberti*). In eastern Poland, very high Pb concentration was found in birds in hunting areas [31]. Zhang et al. [16] found that Pb poisoning increased GRP78 protein expression in rat astrocyte cells. Pb exposure promoted caspase-3 activity in the rat proximal tubular cells [32]. ER stress can induce apoptosis [33]. Therefore, we detected GRP78, GRP94, ATF4, ATF6, IRE, Bcl-2, and caspase-3 mRNA and protein expression and caspase-12 protein expression to investigate Pb-induced apoptosis via ER stress in chicken kidneys.

NO plays roles both in physiological and pathophysiological consequences [34]. NO is produced by iNOS. iNOS catalyzed overproduction of NO [35]. GRP78 was highly upregulated in NO-generating cells [34]. GRP78 and GRP94 are ER chaperonins and play major roles in ER integrity [36]. ATF6 and IRE1 are two of three types of ER membrane receptors and may sense stress in ER and eventually activate transcription factors for induction of GRP78 [37]. ATF6, an ER stress-regulated transmembrane transcription factor, binds GRP78 and dissociate in response to ER stress [38]. ATF4 can activate the GRP78 promoter independent of ER stress elements [39]. ER stress activated ER-resident caspase-12 [40] which may play a role in apoptosis after ER stress [41]. caspase-12 indirectly activated cytoplasmic caspase-3, and finally induced neuronal apoptosis [41]. Bcl-2-related proteins are located on the ER membrane [42]. NO produced by astrocytes after hypoxic insult downregulated Bcl-2, and then activated caspase-3, induced apoptotic death of neurons [43]. In our experiment, we found that excess Pb increased GRP78, GRP94, ATF4, ATF6, IRE, and caspase-3 mRNA and protein expression; increased caspase-12 protein expression; and decreased Bcl-2 mRNA and protein expression in chicken kidneys. Our finding indicated that excess Pb induced ER stress and apoptosis in chicken kidneys. Consistent with our results, other researchers also found that Pb, Cd, and As could induce ER stress and apoptosis. Excess Pb induced GRP78 and GRP94 protein expression, induced ATF4 mRNA expression, and caused ER stress and apoptosis in bovine aortic endothelial cells [44]. Excess Cd upregulated GRP78 and GRP94 mRNA expression, and caused ER stress and apoptosis in chicken hepatocytes [45]. Cd exposure increased NO content; increased iNOS activity; increased iNOS, GRP78, GRP94, ATF4, ATF6, IRE, and caspase-3 mRNA expression; decreased Bcl-2 mRNA expression; and caused ER stress and apoptosis in chicken kidneys [26]. Chen et al. found that excess Cd increased NO content; increased iNOS activity; increased GRP78, ATF6, caspase-12, and caspase-3 mRNA expression; increased caspase-12 protein expression; and caused ER stress and apoptosis in chicken neutrophile granulocytes [27]. Excess As caused apoptosis via ER stress by inducing the mRNA and protein expression of GRP78 and GRP94; inhibiting Bcl-2 protein expression; and increasing caspase-3 and caspase-12 activities in mouse osteoblasts [46]. Our morphological study also demonstrated that Pb poisoning led to apoptosis in chicken kidneys. Other morphological researches were similar with our results. Excess Pb induced apoptosis in chicken kidneys [6] and rat brain hippocampi [47]. In addition, we also found that Pb had time-dependent manners on NO content and iNOS, GRP78, GRP94, ATF4, IRE, and caspase-3 mRNA expression in chicken kidneys. Shinkai et al. [44] also found that Pb increased GRP78 and GRP94 protein expression in a time-dependent manner in bovine aortic endothelial cells.

Se could alleviate Pb and Cd poisoning by decreasing NO, iNOS, GRP78, GRP94, ATF4, ATF6, IRE, caspase-12, and caspase-3 and increasing Bcl-2. Se alleviated the increase of NO content, iNOS activity, and iNOS mRNA and protein expression in chicken testes [48]; decrease of Bcl-2 mRNA and protein expression; increase of caspase-3 mRNA and protein expression; and apoptosis induced by Pb in chicken kidneys [6]. Liu et al. [26] reported that Se alleviated increase of NO content, iNOS activity, and iNOS, GRP78, GRP94, ATF4, ATF6, IRE, and caspase-3 mRNA expression; decrease of Bcl-2 mRNA expression; and ER stress caused by Cd in chicken kidneys. Se also alleviated increase of NO content, iNOS activity, GRP78, ATF6, caspase-12, caspase-3 mRNA expression, and caspase-12 protein expression; and apoptosis via ER stress caused by Cd in the neutrophils of chickens [27]. Se supplementation protected against increase of NO content, iNOS activity, and caspase-3 mRNA expression; decrease of Bcl-2 mRNA expression; and apoptosis caused by Cd in chicken livers [23]. Therefore, we investigated the alleviative effect of Se on Pb-induced apoptosis via ER stress in chicken kidneys. Similar results were obtained in our study. In our experiment, we found that Se alleviated the increase of NO content, iNOS activity, iNOS, GRP78, GRP94, ATF4, ATF6, IRE, caspase-3 mRNA expression, and caspase-12 protein expression and the decrease of Bcl-2 mRNA expression caused by Pb in chicken kidneys. Our results indicated that Se alleviated Pb-induced apoptosis via ER stress in chicken kidneys. The ultrastructural observation of our study also indicated that Se alleviated Pb-induced ultrastructure changes and Pb-induced apoptosis in chicken kidneys. Zhang et al. [23] also found that Se alleviated Cd-induced ultrastructural changes and Cd-induced apoptosis in chicken livers.

Pb pollution led to Pb poisoning in wild birds and even impacted biodiversity of wild birds. Therefore, chickens were used as experimental animals to investigate toxic effect of Pb and alleviative effect of Se on Pb toxicity in our experiment. The results of our experiment indicated that excess Pb caused ultrastructure changes, upregulated NO content, iNOS activity, iNOS, GRP78, GRP94, ATF4, ATF6, IRE, caspase-3 mRNA and protein expression, and caspase-12 protein expression, and downregulated Bcl-2 mRNA and protein expression. Se alleviated all the above changes caused by Pb. There were time-dependent effects on NO content, iNOS, GRP78, GRP94, ATF4, IRE, and caspase-3 mRNA expression. Se alleviated Pb-induced apoptosis via ER stress in chicken kidneys.

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

Compliance with Ethical Standards All procedures used in this experiment were approved by the Northeast Agricultural University's Institutional Animal Care and Use Committee under the approved protocol number SRM-06.

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Conflict of Interest The authors declare that they have no conflict of interest.

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