Biological Trace Element Research (2018) 185:162–169
https://doi.org/10.1007/s12011-017-1200-2

The Antagonistic Effect of Selenium on Lead-Induced Immune Dysfunction via Recovery of Cytokine and Heat Shock Protein Expression in Chicken Neutrophils

Mengyuan Xing[1] · Xi Jin[1] · Jinliang Wang[2] · Qunxiang Shi[1] · Jingzeng Cai[1] · Shiwen Xu[1]

Received: 9 September 2017 / Accepted: 7 November 2017 / Published online: 20 December 2017

Abstract
Lead (Pb) is a ubiquitous and toxic heavy metal and it can damage the immune system in humans and animals. Many researchers have reported that Selenium (Se) could possess various pharmacological effects in mammals. However, few studies have been carried out to investigate the protective role of Se in birds, especially in chickens. In this study, we investigated the protective effects of Se against Pb-induced inflammatory responses and the expression of heat shock proteins (HSPs) in peripheral blood neutrophils. One hundred eighty Hy-Line brown chickens were randomly divided into the control group (Con group), Se supplementation group (+Se group), Pb supplementation group (+Pb group), and the Se and Pb compound group (Se+Pb group). On the 90th day of the experiment, the peripheral blood was collected to extract neutrophils, and then, the levels of HSPs and cytokines were examined. The results showed that, after Pb treatment, the levels of IL-(1β, 1R, 4, 8, 10, and 12β), TGF-β4, and HSP (27, 40, 60, 70, and 90) mRNA were significantly increased and levels of IL-2 and IFN-γ mRNA were decreased compared with those in the control group. Compared with the control group, the protein levels of HSP60 and HSP70 were also increased in the Pb treatment group. Co-administration of Se (1 mg/kg/day) and Pb resulted in a reversal of the Pb-induced cytokine changes in neutrophils accompanied by a significant decrease in HSPs. Our study demonstrated that Pb could decrease the immune function via changing the expression of cytokines and HSPs in chicken neutrophils, but Se could relieve the toxic effect induced by Pb.

Keywords Lead · Selenium · Neutrophil · Chickens · Cytokines · Heat shock proteins

Introduction

Lead (Pb), a highly toxic metal, is commonly found in both biotic and abiotic components in an ecosystem. Currently, possible sources of Pb in the poultry industry include lead solder in food cans and lead-arsenate pesticides, among others [1]. Pb exposure may cause various physiologic changes, affecting hematopoietic, reproductive, vascular, and physiological functions. Additionally, Pb may cause carcinogenesis, mutagenesis, and teratogenesis in experimental animals [2]. In humans, Pb affected different systems and caused neurological symptoms such as headache, lethargy, peripheral neuropathy, severe convulsions, encephalopathy, and even coma [3]. In poultry, broiler chickens are vulnerable to Pb intoxication. Even 1.0 ppm Pb in the diet could cause significant growth suppression and consistent declines in blood D-aminolevulinic acid dehydratase and in an erythrocyte enzyme sensitive to Pb in chickens [1]. Lead can cause adverse effects on the immune system. Pb exacerbates systemic lupus erythematosis (SLE) symptoms in lupus-prone NZM mice [4]. Lead could also affect cells involved in cell-mediated immunity, such as neutrophil leukocytes [5]. Specifically, Pb inhibited in vitro neutrophil chemotaxis, phagocytosis, and superoxide formation and impaired cell chemotaxis [6]. Cytokines are regulatory proteins that play a primary role in modulating innate and adaptive immune responses, including lymphocyte activation, proliferation, differentiation, and

All other authors have read the manuscript and agreed to submit it in its current form for consideration for publication in the journal.

✉ Shiwen Xu
shiwenxu@neau.edu.cn

[1] College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, People's Republic of China

[2] Shandong Binzhou Animal Science & Veterinary Medicine Academy, Binzhou 256600, People's Republic of China

apoptosis. They can be classified as interleukins, such as interleukin-4 (IL-4) and interleukin-1 (IL-1); interferons, such as interferon gamma (IFN-γ); colony-stimulating factors; tumor necrosis factors; tumor growth factors; and chemokines. Cytokines might be affected by heavy metals at levels of exposure that do not affect other constituents of the immune system [7]. A number of cytokines govern neutrophil functions in humans [8]. Heat shock proteins (HSPs), a kind of stress protein that exists in all kinds of cells in organisms, have the ability to modulate cellular anti-stress responses and play a key role in protecting organisms from metal stress [9]. HSP70 is one of the biomarkers of oxidative stress induced by heavy metals. A study found that the expression of HSP27 and HSP70 was increased in chromium-treated mice livers [10].

Selenium (Se) is an essential trace element for organisms. Selenoproteins have a crucial role in the maintenance of cellular redox homeostasis in nearly all tissues and are also involved in thyroid hormone metabolism, inflammation, and immunity [11]. Se is a well-established antioxidant and can prevent or decrease the harmful effects of heavy metal on the antioxidant system in different tissues [12]. The protective effect of Se against the toxicity of different heavy metals in biological systems has been studied [13]. Se can counteract the toxicity of various elements, especially Hg^{2+} and MeHg [14]. Se has been shown to antagonize lead-induced neurotoxicity through the regulation of uptake and excretion of Pb and to prevent damage induced by oxygen free radicals [15]. Supplementation with a suitable dose of Se could alleviate the effects of Pb toxicity in the brain and blood of rats [16]. Moreover, Se is known to coexist with Hg in fish and sea mammals [17] and might play a role in antagonizing MeHg toxicity. Li et al. have already demonstrated the protective effects of Se against sub-chronic exposure to dietary Cd, which could cause hepatotoxicity, oxidative stress, and apoptosis in chicken liver [18]. Ebselen, a seleno-organic compound, could inhibit Mn-induced ROS generation and was efficacious in reducing Mn-induced neuroinflammation, oxidative stress, and locomotor activity impairments [19]. Generally, studies on the antagonism of Se toward toxicity induced by Pb in chickens are mainly focused on its antioxidant function. However, its effects on cytokines and HSP pathways have rarely been reported. Thus, we designed the current experiment to investigate the effects of Pb on the cytokines and HSPs in neutrophils of chicken peripheral blood and to examine the antagonistic effects of Se against lead.

Materials and Methods

Animal Model and Experimental Design

A total of 180 local 1-day-old male Hy-Line brown chickens were randomly divided into four groups (n = 45). Each group was randomly allocated to three pens (15 chickens per pen). During the entire experimental period, the chickens were given free access to food and water. In the present study, we fed chickens a basal diet or a diet supplemented with Pb, Se, or Pb+Se. The control group (Con group) was maintained on a basal diet containing 0.49 mg kg^{-1} Se. The Se-adequate group (+Se group) was maintained on a Se-supplemented basal diet containing 1.00 mg kg^{-1}Se and conventional drinking water, while the Pb-supplemented group (+Pb group) was maintained on a basal diet with 350 mg kg^{-1} lead added to the drinking water. The Se and Pb compound group (Se+Pb group) was maintained on a Se-supplemented basal diet containing 1.00 mg kg^{-1} Se with 350 mg kg^{-1} Pb added to the drinking water. Se was added as sodium selenite, and Pb was added as lead acetate. All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. Chickens were euthanized at 90 days. The whole blood was quickly removed, and the neutrophils were separated immediately and stored at − 80 °C until subsequent experiments.

In this study, the neutrophils were collected at five sampling events, and five chickens per group were used in the official test (n = 5), which was repeated in triplicate. The neutrophils were separated using chicken peripheral blood neutrophil isolation kits according to the manufacturer's instructions (TBD, China).

Primer Design

The primer sequences of cytokines IL-1β, IL-1R, IL-2, IL-4, IL-8, IL-10, IL-12, IFN-γ, and TGF-β4 and of HSPs HSP27, HSP40, HSP60, HSP70, and HSP90 published in GenBank (Table 1) were synthesized by the Invitrogen Biotechnology Co. Ltd. in Shanghai, China. GAPDH was used as an internal reference gene.

Total RNA Isolation and Reverse Transcription

The total RNA was isolated from neutrophil samples using Trizol (Invitrogen, China) according to the manufacturer's instructions. The dried RNA pellets were resuspended in 40 μl of diethylpyrocarbonate-treated water. The concentration and purity of the total RNA was determined spectrophotometrically at 260/280 nm (Gene Quant 1300/100, USA). The total RNA was immediately used to synthesize first-strand cDNA using the AccuPower® RocketScript™RT PreMix (BIONEER) according to the manufacturer's instructions. Reverse transcription (RT) reactions (60 μL) consisted of the following: 6 μL of total RNA, 1.5 μL of RNase inhibitor, 3 μL of dNTP mixture, 3 μL of 20× OligodT(25), 3 μL of Golden MLV Reverse Transcriptase, 6 μL of 10× RT buffer,

Table 1 Gene-special primers used for qPCR

Gene	Primer sequence
IL-1β	Forward 5′-CAGCAGCCTCAGCGAAGAG-3′ Reverse 5′-CTGTGGTGTGCTCAGAATCCA-3′
IL-1R	Forward 5′-AAGTGGTGTGTATGGCTTGCT-3′ Reverse 5′-TGTTCCCTGTAAGTGCTGTCA-3′
IL-2	Forward 5′-GAACCTCAAGAGTCTTACGGGTCTA-3′ Reverse 5′-ACAAAGTTGGTCAGTTCATGGAGA-3′
IL-4	Forward 5′-GTGCCCACGCTGTGCTTAC-3′ Reverse 5′-AGGAAACCTCTCCCTGGATGTC-3′
IL-8	Forward 5′-GGCTTGCTAGGGGAAATGA-3′ Reverse 5′-AGCTGACTCTGACTAGGA AACTGT-3′
IL-10	Forward 5′-CGCTGTCACCGCTTCTTCA-3′ Reverse 5′-TCCCGTTCTCATCCATCTTCTC-3′
IL-12	Forward 5′-TGTCTCACCTGCTATTTGCCTTAC-3′ Reverse 5′-CATACACATTCTCTCTAAGTTTCCACTGT-3′
IL-8	Forward 5′-GGCTTGCTAGGGGAAATGA-3′ Reverse 5′-AGCTGACTCTGACTAGGA AACTGT-3′
IFN-γ	Forward 5′-CATCTTTTTGGGTTAGGCATCC-3′ Reverse 5′-ACTGGATGGCTGGCTTGG-3′
TGF-β4	Forward 5′-ACCTCGACACCGACTACTGCTT-3′ Reverse 5′-ATCCTTGCGGAAGTCGATGT-3′
HSP27	Forward 5′-ACACGAGGAGAAACAGGATGAG-3′ Reverse 5′-ACTGGATGGCTGGCTTGG-3′
HSP40	Forward 5′-GGGCATTCAACAGCATAGA-3′ Reverse 5′-TTCACATCCCCAAGTTTAGG-3′
HSP60	Forward 5′-AGCCAAAGGGCAGAAATG-3′ Reverse 5′-TACAGCAACAACCTGAAGACC-3′
HSP70	Forward 5′-CGGGCAAGTTTGACCTAA-3′ Reverse 5′-TTGGCTCCCACCCTATCTCT-3′
HSP90	Forward 5′-TCCTGTCCTGGCTTTAGTTT-3′ Reverse 5′-AGGTGGCATCTCCTCGGT-3′
GADPH	Forward 5′-AGAACATCATCCCAGCGT-3′ Reverse 5′-AGCCTTCACTACCCTCTTG-3′

and 37.5 μL RNase Free H_2O. The RT procedure was carried out for 15 min at 30 °C followed by 50 min at 55 °C and 10 min at 85 °C. The RT products (cDNA) were diluted five times with sterile water and stored at − 80 °C until use.

Quantitative Real-Time PCR (qPCR)

The qPCR was performed with Light Cycler® 96 (Roche Life Science). Reactions were performed in a 10-μL reaction mixture containing 5 μL of 2× SYBR Green PCR Master Mix (Roche, Switzerland), 1 μL of diluted cDNA, 0.3 μL of each primer (10 μM), and 3.4 μL of PCR-grade water. The qPCR procedure for HSPs, inflammatory genes, and β-actin consisted of heating the reaction mixture to 52 °C for 2 min and 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s, and 60 °C for 20 s. The melting curve analysis showed only one peak for each PCR product.

Western Blot Analysis

Protein extracts were subjected to SDS-polyacrylamide gel electrophoresis under reducing conditions on 12% gels. Separated proteins were then transferred to nitrocellulose membranes using a tank transfer for 60 min at 200 mA in Tris-glycine buffer containing 20% methanol. Membranes were blocked with 5% skim milk for 16–24 h and incubated overnight with the diluted primary chicken antibodies HSP60 and HSP70(1:1000, production of polyclonal antibody by our lab) followed by a horseradish peroxidase (HRP)-conjugated secondary antibody against rabbit IgG (1:2000, Santa Cruz, CA, USA). To verify equal loading of samples, the membrane was incubated with monoclonal β-actin antibody (1:1000, Santa Cruz, CA, USA) followed by a HRP-conjugated goat anti-mouse IgG (1:2000). The signal was detected using X-ray films (Trans Gen Biotech Co., China). The optical density (OD) of each band was determined using the Image VCD gel imaging system, and iNOS expression was detected as the OD ratio of HSP60 and HSP70 to β-actin.

Statistical Analysis

Statistical analysis of all data was performed using SPSS for Windows (version 19, SPSS Inc., USA). The data was analyzed using a one-way analysis of variance, and measurements were considered significantly different when $P < 0.05$. All data were checked for normal distribution and equal variance. Differences between means were assessed using Tukey's honest significant difference test for post hoc multiple comparisons. The data were expressed as the mean ± standard deviation. All experiments were performed a minimum of three times. In addition, principal component analysis (PCA) was used to define the most important parameters, which could be used as key factors for individual variations using the Statistics 6.0 program (version 19, SPSS Inc., Chicago, IL, USA).

Results

Effects of Se, Pb, and Se+Pb on the mRNA Levels of Cytokines in Chicken Neutrophils

The mRNA levels of cytokines (IL-1β, IL-1R, IL-2, IL-4, IL-8, IL-10, IL-12, IFN-γ, and TGF-β4) in chicken neutrophils were measured by RT-PCR (Fig. 1). Compared with the control group, the mRNA expression levels of IL-1β, IL-1R, IL-4, IL-8, IL-10, IL-12, and TGF-β4 were significantly increased ($P < 0.05$) in the +Pb group, and significant decreases were also observed in the mRNA levels of IL-2 and IFN-γ of the +Pb group ($P < 0.05$). However, compared with the +Pb group, treatment with Se significantly reduced the mRNA

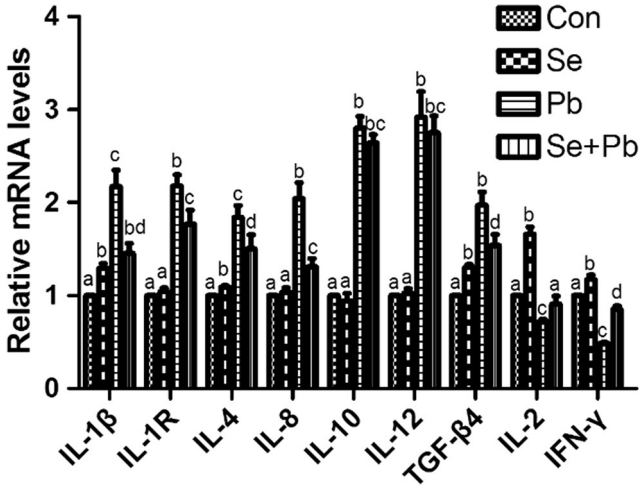

Fig. 1 Effects of dietary Se and Pb on the mRNA levels of cytokines in chicken neutrophils. The relative mRNA expression levels compared to the control group were used as the reference values. Each value is represented as the mean ± SD ($n = 5$). Bars not sharing a common letter indicate that there were significant differences between the two groups ($P < 0.05$)

expression of IL-1β, IL-1R, IL-4, IL-8, IL-10, IL-12, and TGF-β4 and increased the mRNA expression of IL-2 and IFN-γ in the Se+Pb group ($P < 0.05$).

Effects of Se and Pb on the mRNA Levels of HSPs in Chicken Neutrophils

The mRNA levels of HSPs in chicken neutrophils were detected by RT-PCR (Fig. 2). Compared with the control groups, the mRNA levels of HSP27, HSP40, HSP60, HSP70, and HSP90 were significantly higher ($P < 0.05$) in the +Pb group. However, compared to the +Pb group, there was a significant decrease in the mRNA levels of HSPs in the Se+Pb group ($P < 0.05$), though the values were not restored to the levels of the control group.

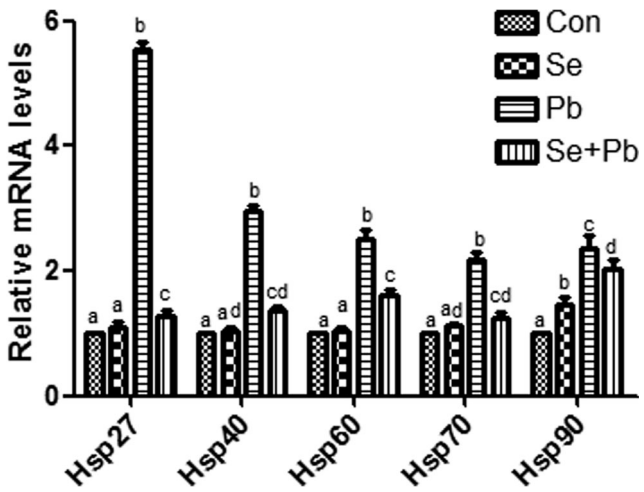

Fig. 2 Effects of Se and Pb on the mRNA level of HSP genes in chicken neutrophils. The relative mRNA expression levels compared to the control group were used as the reference values. Each value is represented as the mean ± SD ($n = 5$). Bars not sharing a common letter indicate that there were significant differences between the two groups ($P < 0.05$)

Effects of Se and Pb on the Protein Levels of HSPs in Chicken Neutrophils

The protein levels of HSP60 and HSP70 in chicken neutrophils were examined using Western blots (Fig. 3). The results showed that, compared with the control group, Pb treatment significantly increased the protein expression of HSP60 and HSP70 ($P < 0.05$). However, compared with the +Pb group, Se supplementation notably reduced these protein levels in the Se+Pb group, though the values were not restored to the levels of the control group ($P > 0.05$).

Principal Component Analysis

Using PCA, nine cytokines (IL-1β, IL-1R, IL-2, IL-4, IL-8, IL-10, IL-12, IFN-γ, and TGF-β4) and five heat shock proteins (HSP27, HSP40, HSP60, HSP70, and HSP90) from four treatment groups of chicken neutrophils were analyzed. A 3D plot of the PCA loadings is presented in Fig. 4 that clearly indicates correspondence to the first, second, and third principal components (86.153, 6.546, and 4.918%, respectively) in chicken neutrophils.

As shown in Table 2, all the cytokines and heat shock proteins had closer relationships with the first principal components. The results revealed that HSP40, HSP60, HSP70, and HSP90 had a close positive correlation with each other in chicken neutrophils. Furthermore, of all the parameters that were measured in the present study, only IL-2 and IFN-γ had negative correlations with another parameter, while IL-1β, IL-1R, IL-4, IL-8, IL-10, IL-12β, TGF-β4, HSP27, HSP40, HSP60, HSP70, and HSP90 had close positive correlations with each other in chicken neutrophils.

Discussion

Pb is a common industrial poison and an environmental pollutant. A great deal of experimental data has suggested that acute and chronic exposure to inorganic lead may result in the impairment of immune functions in animals. For example, Pb triggered humoral and cellular immune response depressions in rodents [20]. Neutrophil, a type of phagocyte, is an essential part of the innate immune system. A previous study has demonstrated that lead exposure could inhibit neutrophil function [21]. Cytokines are effectors in the immune system that mediate activating and regulatory functions, which are important for immune responses [22]. Interleukins are a group of cytokines that are critical to the functioning of the immune system. Evidence suggests that the abnormal expression of

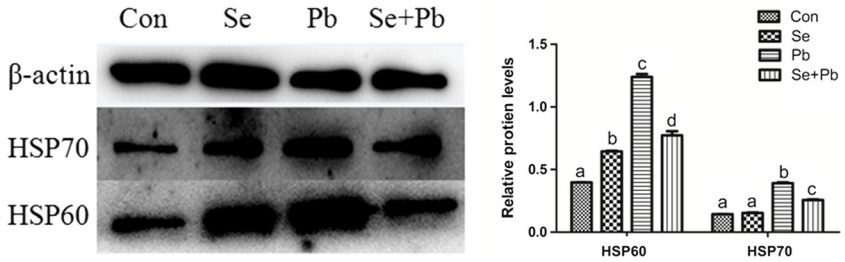

Fig. 3 Effects of Se and Pb on the protein level of HSP genes in chicken neutrophils. The relative protein expression levels compared to the control group were used as the reference values. Each value is represented the mean ± SD ($n = 5$). Bars not sharing a common letter indicate that there were significant differences between the two groups ($P < 0.05$)

interleukins could be induced by heavy metals and might impair immune function. For instance, IL-1R is involved in multiple immunological and inflammatory processes [23]. IL-1β is considered as an acute phase reactant; it is associated with fever, inflammation, tissue destruction, and occasionally even shock and death as an endogenous pyrogen [24]. Xu reported that cadmium caused a significant increase in IL-1β in chicken spleen lymphocytes [25]. IL-2 is related to the proliferation of B cells and enhances cytokine production of natural killers and neutrophils. It has been reported that suppressed IL-2 levels were associated with a general decline in immune function and immune regulation [26]. Liu X et al. has reported that the mRNA expression of IL-2 was also suppressed by manganese in Hy-Line brown cocks [27]. Total IL-4 is a known marker for humoral immune responses [28]. IL-8 is a member of the CXC chemokine subfamily. Neutrophils are major specific targets for IL-8 action, and the pathophysiological actions of IL-8 depend on the activation of neutrophils [29]. Pb toxicity affects the body systems by inducing changes in IL-8 [30]. Lin et al. proved that Pb^{2+} induced IL-8 gene expression by extracellular signal-regulated kinases [31]. IL-10 inhibits monocyte/macrophage and neutrophil generation and promotes a shift from the Th1 phenotype to the Th2 phenotype in T lymphocytes [32]. Villanueva et al. confirmed that Pb ions could inhibit the activities of Th1 cells but could increase the activity of Th2 cells [33]. IL-4 and IL-10 are secreted by Th2 cells, and Pb ions increase the expression of IL-4 and IL-10. IL-12 is a primary cytokine secreted by antigen-presenting cells. TGF-β expression inhibits IFN-γ generation in human vascular cells [34]. TGF-β4 is produced by Treg cells converted from CD4+Th17 cells [35]. TGF-β mRNA expression was significantly increased in the kidney of Chinook salmon exposed to CPF. Parenthetically, it has been observed that TGF-β4 mRNA significantly increases in the duodenum of chickens infected with *Eimeria acervulina* [36]. Finally, IFN-γ activates neutrophils, NK cells, and vascular endothelial cells. The role of IFN-γ in mediating the cellular immune response has been identified [37]. Kaminska et al.

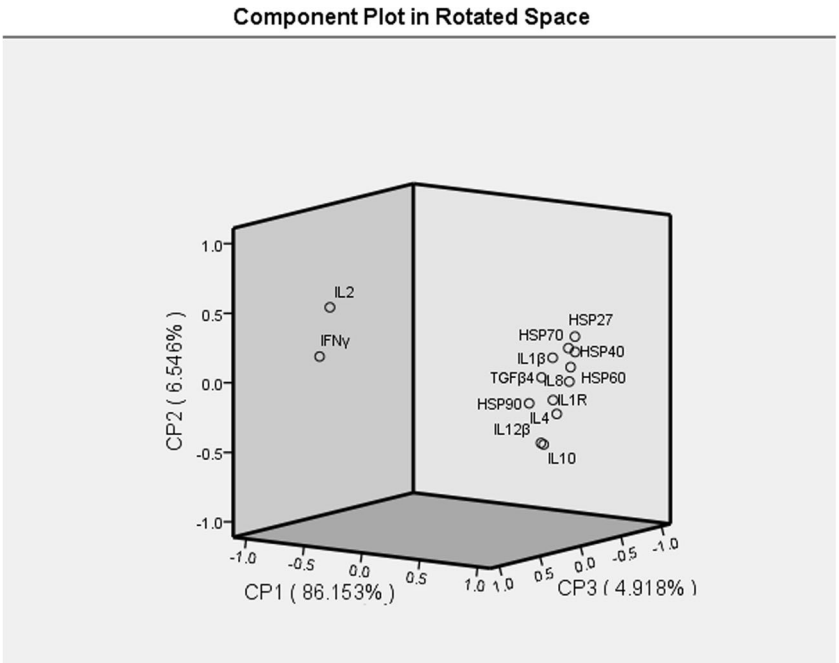

Fig. 4 Principal component analysis of IL-1β, IL-1R, IL-2, IL-4, IL-8, IL-10, IL-12, IFN-γ, TGF-β4, HSP27, HSP40, HSP60, HSP70, and HSP90

Table 2 Correlation matrix

	IL-1β	IL-1R	IL-2	IL-4	IL-8	IL-10	IL-12β	IFN-γ	TGF-β4	HSP27	HSP40	HSP60	HSP70	HSP90
IL-1β	1	0.87	−0.498	0.915	0.958	0.794	0.785	−0.854	0.953	0.917	0.95	0.963	0.927	0.888
IL-1R	0.87	1	−0.707	0.931	0.904	0.947	0.935	−0.922	0.873	0.806	0.872	0.932	0.862	0.948
IL-2	−0.498	−0.707	1	−0.638	−0.649	−0.745	−0.719	0.841	−0.494	−0.58	−0.627	−0.69	−0.562	−0.494
IL-4	0.915	0.931	−0.638	1	0.89	0.915	0.922	−0.892	0.933	0.824	0.877	0.953	0.89	0.889
IL-8	0.958	0.904	−0.649	0.89	1	0.815	0.8	−0.935	0.91	0.945	0.977	0.98	0.936	0.852
IL-10	0.794	0.947	−0.745	0.915	0.815	1	0.979	−0.852	0.851	0.658	0.748	0.879	0.725	0.903
IL-12β	0.785	0.935	−0.719	0.922	0.8	0.979	1	−0.839	0.861	0.657	0.741	0.867	0.734	0.893
IFN-γ	−0.854	−0.922	0.841	−0.892	−0.935	−0.852	−0.839	1	−0.815	−0.896	−0.934	−0.948	−0.901	−0.783
TGF-β4	0.953	0.873	−0.494	0.933	0.91	0.851	0.861	−0.815	1	0.843	0.886	0.94	0.882	0.9
HSP27	0.917	0.806	−0.58	0.824	0.945	0.658	0.657	−0.896	0.843	1	0.989	0.924	0.979	0.739
HSP40	0.95	0.872	−0.627	0.877	0.977	0.748	0.741	−0.934	0.886	0.989	1	0.962	0.978	0.808
HSP60	0.963	0.932	−0.69	0.953	0.98	0.879	0.867	−0.948	0.94	0.924	0.962	1	0.941	0.881
HSP70	0.927	0.862	−0.562	0.89	0.936	0.725	0.734	−0.901	0.882	0.979	0.978	0.941	1	0.806
HSP90	0.888	0.948	−0.494	0.889	0.852	0.903	0.893	−0.783	0.9	0.739	0.808	0.881	0.806	1

have proven that leukocyte production of IFN-γ in vitro could be significantly reduced by different concentrations of lead in cows [38]. It has been documented that lead could inhibit the Th type 1 (Th1) response, which is characterized by IL-2 and IFN-γ secretion and the promotion of cellular cytotoxic immunity [7]. In the present study, we also detected expression of other cytokines. These results showed that Pb treatment significantly increased the expression of IL-1β, IL-1R, IL-4, IL-8, IL-10, IL-12, and TGF-β4 and decreased that of IL-2 and IFN-γ, which is consistent with the previous studies. It suggested that Pb treatment could induce neutrophil injury and thereby impair immune function in chickens.

HSPs are a family of proteins that are produced by cells in response to exposure to stressful conditions, including heavy metal exposures [39]. The level of HSP mRNA (HSP27, HSP40, HSP60, HSP70, and HSP90) was significantly increased in the immune organs when chickens were exposed to arsenic trioxide [40]. Pb poisoning also induced mRNA expression of HSPs (HSP27, HSP40, HSP60, HSP70, and HSP90) in chicken peripheral blood lymphocytes [41]. Pb administration in Wistar rats also increased expression of HSP70 in the brain [42]. The increased expression of HSP90 has been reported to be a major mechanism for Pb resistance [43]. Consistent with the previous studies, our results showed that the mRNA for the five HSPs (HSP27, HSP40, HSP60, HSP70, and HSP90) was upregulated. It elucidated that Pb could also have an adverse influence on neutrophils by increasing the mRNA levels of HSPs to further impair the immune system of chickens.

Se plays an important biological role in humans and animals, including in immune function enhancement, by increasing the number of cytokines, such as interferon. For example, Seda et al. reported that Se at 0.625 mg/kg/day decreased IL-1β levels during the treatment of chemia-reperfusion-induced brain injury in rats [44]. The results agreed with Montgomery et al., who suggested that Se might enhance the immunity of young foals through increasing the expression of IL-2 [45]. Additionally, Se could also antagonize the toxic effects of heavy metals [46, 47]. Se could also ameliorate cadmium (Cd)-induced brain damage in chickens [48] and exhibited protective effects on the chronic poisoning and decreased HSP70 mRNA levels induced by arsenic in rat livers [49]. Se exhibited significant antagonistic roles against Pb-induced increases of HSP expression in chicken liver [50].

In the current study, with Se supplementation, the increased levels of IL-(1β, 1R, 4, 8, 10, and 12) and TGF-β4 mRNA and the decreased levels of IL-2 and IFN-γ mRNA induced by Pb were returned nearly to the levels seen in the control group. The increased levels of HSPs induced by Pb were similarly mitigated by Se supplementation. Our results were in agreement with previous studies. They illustrated that Se could antagonize Pb toxicity to recover the immune system function by affecting the expression of the cytokines IL-(1β, 1R, 2, 4, 8, 10, and 12), IFN-γ, and TGF-β4 and of HSP (27, 40, 60, 70, and 90).

As shown in the results of the PCA, there is a correlation between nine cytokines and the five HSPs. IL-2 and IFN-γ are negatively correlated with other parameters, but there is a positive correlation between IL-(1β, 1R, 4, 8, 10, and 12), TGF-β4, and HSP (27, 40, 60, 70, and 90). However, the correlations between cytokines and heat shock proteins were different. For example, IL-1β is highly related to HSP40, HSP60, and HSP70, but the correlation between IL-2 and HSP90 is small. Thus, we suggest that Pb poisoning not only affects the expression of cytokines and HSPs but also affects the expression in the two genes.

In conclusion, Pb could increase the expression of IL-(1β, 1R, 4, 8, 10, and 12) and TGF-β4, decrease that of IL-2 and IFN-γ, and increase that of HSP (27, 40, 60, 70, and 90) to induce neutrophil injury and thereby impair the immune system of chickens. However, Se could antagonize Pb toxicity in neutrophils in chickens.

Funding Information This study was supported by the National Natural Science Foundation of China (31272626) and the International (Regional) Cooperation and Exchange Projects of the National Natural Science Foundation of China (31320103920).

Compliance with Ethical Standards All procedures used in this study were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University.

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

References

1. Bakalli RI, Pesti GM, Ragland WL (1995) The magnitude of lead toxicity in broiler chickens. Vet Hum Toxicol 37(1):15–19
2. Beulke SH (1999) Casarett and Doull's toxicology the basic science of poisons, fifth edition companion handbook. Aust J Hosp Pharm 29(1):74–74. https://doi.org/10.1002/jppr199929174
3. Ciobanu C, Slencu BG, Cuciureanu R (2012) Estimation of dietary intake of cadmium and lead through food consumption. Rev Med Chir Soc Med Nat Iasi 116(2):617–623
4. Hudson C, Cao L, KastenJolly J, Kirkwood J, Lawrence D (2003) Susceptibility of lupus-prone Nzm mouse strains to lead exacerbation of systemic lupus erythematosus symptoms. J Toxicol Environ Health A 66(10):895–918. https://doi.org/10.1080/15287390306456
5. Queiroz ML, Almeida M, Gallão MI, Höehr NF (1993) Defective neutrophil function in workers occupationally exposed to lead. Basic Clin Pharmacol Toxicol 72(2):73–77. https://doi.org/10.1111/j.1600-0773.1993.tb00293.x
6. Governa M, Valentino M, Visonà I (1987) In vitro impairment of human granulocyte functions by lead. Arch Toxicol 59(6):421–425. https://doi.org/10.1007/BF00316208
7. Shen X, Lee K, König R (2001) Effects of heavy metal ions on resting and antigen-activated CD4 + T cells. Toxicology 169(1):67–80. https://doi.org/10.1016/S0300-483X(01)00483-8
8. Binder R, Kress A, Kan G, Herrmann K, Kirschfink M (1999) Neutrophil priming by cytokines and vitamin D binding protein (Gc-globulin): impact on C5a-mediated chemotaxis, degranulation and respiratory burst. Mol Immunol 36(13–14):885–892. https://doi.org/10.1016/S0161-5890(99)00110-8
9. Chen H, Xu XL, Li YP, Wu JX (2014) Characterization of heat shock protein 90, 70 and their transcriptional expression patterns on high temperature in adult of Grapholita molesta (Busck). Insect Sci 21(4):439–448. https://doi.org/10.1111/1744-7917.12057
10. Lee J, Lim KT (2012) Inhibitory effect of SJSZ glycoprotein (38 kDa) on expression of heat shock protein 27 and 70 in chromium (VI)-treated hepatocytes. Mol Cell Biochem 359(1–2):45–57. https://doi.org/10.1007/s11010-011-0998-8
11. Yao HD, Wu Q, Zhang ZW, Zhang JL, Li S, Huang JQ, Ren FZ, SW X, Wang XL, Lei XG (2013) Gene expression of endoplasmic reticulum resident selenoproteins correlates with apoptosis in various muscles of se-deficient chicks. J Nutr 143(5):613–619. https://doi.org/10.3945/jn.112.172395
12. Yao HD, Wu Q, Zhang ZW, Li S, Wang XL, Lei XG, Xu SW (2013) Selenoprotein W serves as an antioxidant in chicken myoblasts. Biochim Biophys Acta 1830(4):3112–3120. https://doi.org/10.1016/j.bbagen.2013.01.007
13. Orun I, Talas ZS, Ozdemir I, Alkan A, Erdogan K (2008) Antioxidative role of selenium on some tissues of (Cd2+, Cr3+)-induced rainbow trout. Ecotoxicol Environ Saf 71(1):71–75. https://doi.org/10.1016/j.ecoenv.2007.07.008
14. Hiroshi S, Noriko Y, Satoshi S (1985) Development of reflexes in neonatal mice prenatally exposed to methylmercury and selenite. Toxicol Lett 25(2):199–203. https://doi.org/10.1016/0378-4274(85)90082-7
15. Flora SJ, Singh S, Tandon SK (1983) Role of selenium in protection against lead intoxication. Basic Clin Pharmacol Toxicol 53(1):28–32
16. Lai J, Yin S, Xu Q, Hu S (2004) Protection of zinc and selenium content of brain and blood on learning and memory exposed to lead in growing rats. J Hyg Res 33(2):218
17. Cabañero AI, Carvalho C, Madrid Y, Batoréu C, Cámara C (2005) Quantification and speciation of mercury and selenium in fish samples of high consumption in Spain and Portugal. Biol Trace Elem Res 103(1):17–35. https://doi.org/10.1385/BTER:103:1:017
18. Li JL, Jiang CY, Li S, Xu SW (2013) Cadmium induced hepatotoxicity in chickens (Gallus domesticus) and ameliorative effect by selenium. Ecotoxicol Environ Saf 96(8):103–109. https://doi.org/10.1016/j.ecoenv.2013.07.007
19. Santos AP, Lucas RL, Andrade V, Mateus ML, Milatovic D, Aschner M, Batoreu MC (2012) Protective effects of ebselen (Ebs) and para-aminosalicylic acid (PAS) against manganese (Mn)-induced neurotoxicity. Toxicol Appl Pharmacol 258(3):394–402. https://doi.org/10.1016/j.taap.2011.12.003
20. Kimmel CA (1979) Effect of chronic developmental lead exposure on cell-mediated immune functions. Clin Exp Immunol 35(3):413–420
21. Ündeğer Ü, Başaran N (1998) Effects of lead on neutrophil functions in occupationally exposed workers. Environ Toxicol Pharmacol 5(2):113–117. https://doi.org/10.1016/S1382-6689(97)10066-7
22. Romagnani S (2000) T-cell subsets (Th1 versus Th2). Ann Allergy Asthma Immunol 85(1):9–18. https://doi.org/10.1016/S1081-1206(10)62426-X
23. Verstrepen L, Bekaert T, Chau TL, Tavernier J, Chariot A, Beyaert R (2008) TLR-4, IL-1R and TNF-R signaling to NF-kB: variations on a common theme. Cell Mol Life Sci 65(19):2964–2978. https://doi.org/10.1007/s00018-008-8064-8
24. Giraldo S, Sanchez J, Felty Q, Roy D (2009) IL1B (interleukin 1, beta). Atlas Genet Cytogenet Oncol Haematol 13(4):273–275
25. Xu F, Shuang L, Shu L (2015) Effects of selenium and cadmium on changes in the gene expression of immune cytokines in chicken splenic lymphocytes. Biol Trace Elem Res 165(2):1–8
26. Malek TR, Yu A, Zhu L, Matsutani T, Adeegbe D, Bayer AL (2008) IL-2 family of cytokines in T regulatory cell development and homeostasis. J Clin Immunol 28(6):635–639. https://doi.org/10.1007/s10875-008-9235-y
27. Liu X, Li Z, Han C, Zhang Z, Xu S (2012) Effects of dietary manganese on Cu, Fe, Zn, Ca, Se, IL-1β, and IL-2 changes of immune organs in cocks. Biol Trace Elem Res 148(3):336–344. https://doi.org/10.1007/s12011-012-9377-x
28. Overbergh L, Valckx D, Waer M, Mathieu C (1999) Quantification of murine cytokine mRNAs using real time quantitative reverse transcriptase PCR. Cytokine 11(4):305–312. https://doi.org/10.1006/cyto.1998.0426
29. Bickel M (1993) The role of interleukin-8 in inflammation and mechanisms of regulation. J Periodontol 64(5 Suppl):456–460

30. Yang Y, Zhang X, Fu Y, Yang H (2014) Leptin and IL-8: two novel cytokines screened out in childhood lead exposure. Toxicol Lett 227(3):172–178. https://doi.org/10.1016/j.toxlet.2014.03.009
31. Lin YC, Wei PL, Tsai YT, Wong JH, Chang CM, Wang JY, Hou MF, Lee YC, Chuang HY, Chang WC (2015) Pb^{2+} induced IL-8 gene expression by extracellular signal-regulated kinases and the transcription factor, activator protein 1, in human gastric carcinoma cells. Environ Toxicol 30(3):315–322
32. Cuneo AA, Autieri MV (2009) Expression and function of anti-inflammatory interleukins: the other side of the vascular response to injury. Curr Vasc Pharmacol 7(3):267–276. https://doi.org/10.2174/157016109788340721
33. Villanueva MBG, Koizumi S, Jonai H (2000) Cytokine production by human peripheral blood mononuclear cells after exposure to heavy metals. J Health Sci 46(5):6–21
34. Lebastchi AH, Khan SF, Qin L, Li W, Zhou J, Hibino N, Yi T, Rao DA, Pober JS, Tellides G (2011) TGF-β expression by human vascular cells inhibits IFN-γ production and arterial media injury by alloreactive memory T cells. Am J Transplant Off J Am Soc Transplant Am Soc Transplant Surg 11(11):2332–2341. https://doi.org/10.1111/j.1600-6143.2011.03676.x
35. Cao AT, Yao S, Gong B, Elson CO, Cong Y (2012) Th17 cells upregulate polymeric Ig receptor and intestinal IgA and contribute to intestinal homeostasis. J Immunol 189(9):4666–4673. https://doi.org/10.4049/jimmunol.1200955
36. Jakowlew SB, Mathias A, Lillehoj HS (1997) Transforming growth factor-β isoforms in the developing chicken intestine and spleen: increase in transforming growth factor-β4 with coccidia infection. Vet Immunol Immunopathol 55(4):321–339. https://doi.org/10.1016/S0165-2427(96)05628-0
37. Sen GC (2001) Viruses and interferons. Annu Rev Microbiol 55(1):255–281. https://doi.org/10.1146/annurev.micro.55.1.255
38. Kamińska T, Filar J, Madej E, Szusterciesielska A, Kandeferszerszeń M (1998) Modification of bovine interferon and tumor necrosis factor production by lead in vivo and in vitro. Arch Immunol Ther Exp 46(5):323
39. Sherman M, Multhoff G (2007) Heat shock proteins in cancer. Ann N Y Acad Sci 1113(1):192–201. https://doi.org/10.1196/annals.1391.030
40. Guo Y, Zhao P, Guo G, Hu Z, Li T, Zhang K, Sun Y, Zhang X, Zhang W, Xing M (2016) Effects of arsenic trioxide exposure on heat shock protein response in the immune organs of chickens. Biol Trace Elem Res 169(1):134–141. https://doi.org/10.1007/s12011-015-0389-1
41. Sun GX, Chen Y, Liu CP, Li S, Fu J (2016) Effect of selenium against lead-induced damage on the gene expression of heat shock proteins and inflammatory cytokines in peripheral blood lymphocytes of chickens. Biol Trace Elem Res 172(2):474–480. https://doi.org/10.1007/s12011-015-0602-2
42. Chander K, Vaibhav K, Ahmed ME, Javed H, Tabassum R, Khan A, Kumar M, Katyal A, Islam F, Siddiqui MS (2014) Quercetin mitigates lead acetate-induced behavioral and histological alterations via suppression of oxidative stress, Hsp-70, Bak and upregulation of Bcl-2. Food Chem Toxicol 68(25–26):297–306. https://doi.org/10.1016/j.fct.2014.02.012
43. Li P, Rossman TG (2001) Genes upregulated in lead-resistant glioma cells reveal possible targets for lead-induced developmental neurotoxicity. Toxicol Sci 64(1):90–99. https://doi.org/10.1093/toxsci/64.1.90
44. Özbal S, Erbil G, Koçdor H, Tuğyan K, Pekçetin Ç, Özoğul C (2008) The effects of selenium against cerebral ischemia-reperfusion injury in rats. Neurosci Lett 438(3):265–269. https://doi.org/10.1016/j.neulet.2008.03.091
45. Montgomery JB, Wichtel JJ, Wichtel MG, Mcniven MA, Mcclure JT, Markham F, Horohov DW (2012) Effects of selenium source on measures of selenium status and immune function in horses. Can J Vet Res 76(4):281
46. Xi J, Zhe X, Xia Z, Chen M, Xu S (2017) The antagonistic effect of selenium on lead-induced apoptosis via mitochondrial dynamics pathway in the chicken kidney. Chemosphere 180:259–266
47. Zheng C, Fan W, Xiu C, Jian Z, Li Y (2017) Hypericum perforatum extract attenuates behavioral, biochemical, and neurochemical abnormalities in aluminum chloride-induced Alzheimer's disease rats. Biomed Pharmacother 91:931
48. Liu LL, Zhang JL, Zhang ZW, Yao HD, Sun G, SW X (2014) Protective roles of selenium on nitric oxide-mediated apoptosis of immune organs induced by cadmium in chickens. Biol Trace Elem Res 159(1–3):199–209. https://doi.org/10.1007/s12011-014-0007-7
49. Xu Z, Wang Z, Li JJ, Chen C, Zhang PC, Dong L, Chen JH, Chen Q, Zhang XT, Wang ZL (2013) Protective effects of selenium on oxidative damage and oxidative stress related gene expression in rat liver under chronic poisoning of arsenic. Food Chem Toxicol 58(7):1–7. https://doi.org/10.1016/j.fct.2013.03.048
50. Wang H, Li S, Teng X (2015) The antagonistic effect of selenium on lead-induced inflammatory factors and heat shock proteins mRNA expression in chicken livers. Biol Trace Elem Res 171(2):437–444. https://doi.org/10.1007/s12011-015-0532-z