Biol Trace Elem Res (2015) 168:252–260
DOI 10.1007/s12011-015-0354-z

Protective Roles of Selenium on Nitric Oxide and the Gene Expression of Inflammatory Cytokines Induced by Cadmium in Chicken Splenic Lymphocytes

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Received: 26 February 2015 / Accepted: 23 April 2015 / Published online: 5 May 2015

Abstract Cadmium (Cd) is an environmental toxicant and an inflammation-related xenobiotic. Selenium (Se) is a well-known nutritional trace element and a potent chemopreventive agent. The present study aimed to investigate the effect of Se on the cytotoxicity of Cd in bird immunocytes in vitro. Chicken splenic lymphocytes exposed to $CdCl_2$ (10^{-6} mol/L), Na_2SeO_3 (10^{-7} mol/L), or a mixture of the two (10^{-7} mol/L Na_2SeO_3 and 10^{-6} mol/L $CdCI_2$) were incubated for 12, 24, 36, 48, or 60 h. Cd significantly increased ($P<0.05$ or $P<0.01$) the messenger RNA (mRNA) expression levels of nuclear factor kappaB (NF-κB), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), tumor necrosis factor (TNF-α), and prostaglandin E_2 (PGE_2), and similar results were observed in the protein expression levels of NF-κB and COX-2. In addition, the nitric oxide (NO) content and the inducible iNOS activity were increased in the Cd-treated group compared to the control group. Furthermore, the protective effects of Se against Cd toxicity in chicken splenic lymphocytes were illustrated by the increase in select cytokines (NF-κB, iNOS, COX-2, TNF-α, and PGE_2), NO content and iNOS activity. The biochemical parameters exhibited sensitivity to Se and Cd, suggesting that they may act as potential biomarkers for assessing the effects of Se and Cd risk on chicken splenic lymphocytes.

Keywords Cadmium · Selenium · Inflammation · Cytokines · Nitric oxide · Chicken splenic lymphocytes

Introduction

Cd is an environmental risk factor for osteoporosis, nephrotoxicity, and hepatotoxicity [1]. It is known that one of the primary targets of Cd is the immune system [2]. In experimental studies of mammals, immune function assays have demonstrated that Cd affects the immune system by suppressing lymphocyte proliferation and hemagglutination [3]. Some studies have demonstrated that Cd can induce oxidative stress damage, autophagy, and apoptosis of lymphocytes [2, 4]. In fowl, exposure to Cd results in oxidative damage to the chicken immune system by altering antioxidant defense enzyme systems and leading to increased lipid peroxidation values [5]. The toxic effects of Cd also include a reduction of egg production, kidney damage, testicular damage, and alterations in the behavioral response of birds [6]. It was shown that seabirds, such as the black-tailed godwit (*Limosa limosa*), the lesser scaup (*Aythya affinis*), and the tree sparrow, are susceptible to Cd that entered their food chain, causing potential adverse health effects [7, 8]. However, no information is available regarding the effect of inflammation on the splenic lymphocytes of chickens due to Cd.

Inflammation plays a crucial role in the host defense against invasion of microbial pathogens and is essential for the successful healing of tissue damage [9]. Despite the benefits of inflammation in protecting the host from exogenous and endogenous insults, untimely and unnecessarily high degrees of inflammation can cause host tissue damage.

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

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Therefore, the molecular networks that control the initiation, magnitude, and resolution of inflammation must be properly tuned for the maintenance of homeostasis and the optimization of the host response [10]. Some data have shown that immune cell inflammation is closely related to the expression of inflammatory cytokines. As an important biomarker, excessive production of TNF-α can cause tissue damage in immune organs [11]. NF-κB is a protein complex that can be activated by TNF-α and represents an important nuclear transcription factor, by playing a critical role in inflammation [12]. At the same time, NF-κB also activates the pro-inflammatory genes that encode iNOS and COX-2, leading to an increased synthesis of PGE_2 and NO, which contribute to pro-inflammatory responses [13–15]. It has been demonstrated that NO is an important regulatory molecule for diverse physiological functions, and it plays an important role in the toxicity of heavy metals [16]. Some studies have indicated that Cd toxicity is associated with the massive release of NO, which may play an important role in inducing malfunction of many organs [17, 18]. Liu et al. also showed that the overproduction of NO contributed to Cd-induced immunotoxicity and apoptosis in the immune tissues of chicken [19].

Se is an important nutritional trace element and is well-known as a potent chemopreventive agent [20]. Numerous studies have reported that Se can protect mammals and poultry both in vitro and in vivo against Cd toxicity [21, 22]. According to the studies of Zwolak and Zaporowska, Se reduced Cd accumulation, resulting in a subsequent reduction of its toxicity in the body [23]. Li et al. have already demonstrated the protective effects of Se against subchronic exposure to dietary Cd that otherwise could cause hepatotoxicity, oxidative stress, and apoptosis in chicken liver [24]. Some reports clarified the great importance of Se for human health, since it protects cells from the harmful effects of free radical production, and its deficiency may be related with certain diseases [25]. Se inhibits the activation of transcription factor NF-κB and suppresses the expression of COX-2 and iNOS, which is induced by many pro-inflammatory stimuli, such as lipopolysaccharides (LPS), TNF-α, and ovalbumin [26]. Although Se levels are associated with many inflammatory diseases, the mechanisms responsible for the protective effect of Se against the toxicity of Cd in bird immunocytes and especially in inflammation remains unclear.

Based on the previous findings, we sought to investigate the role of Se in Cd-related toxicology, as Se supplements could be a potential agent to protect birds suffering caused by Cd-induced inflammation. To provide a better understanding of the possible mechanisms of Cd toxicity and the protective effects of Se against Cd-induced immune cell toxicity in birds, cultured cells originating from chicken splenic lymphocytes were used as a model, and the effects of Cd on the expression levels of inflammatory factors (iNOS, NF-κB, PGE_2, COX-2, TNF-α, and NO) were examined.

Materials and Methods

Preparation of Chicken Splenic Lymphocytes Suspension and Treatment

All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. Spleens were aseptically collected from Isa brown cocks (60 days old) and placed in sterile phosphate-buffered saline (PBS, 0.1 M phosphate buffer with 0.85 % NaCl, pH 7.2). Single-cell suspensions were prepared by gently pushing the splenic pulp through a sterile stainless steel mesh with a pore size of 100 μM. The cells were washed and resuspended in 5 mL of sterile PBS and then layered over 5 mL of lymphocyte separation medium (Tian Jin Hao Yang Biological Manufacture Co. Ltd, China). The splenocyte preparations were enriched by centrifugation (2000×*g*) for 15 min at 18 °C. The cells were recovered from the interface, resuspended, and washed twice in 8 mL of cell culture medium (RPMI 1640, Gibco, USA). The cells were suspended in complete cell culture medium [RPMI 1640 containing HEPES and 2 mM glutamine, supplemented with 10 % fetal bovine serum (FBS, Gibco, USA) and 1 % antibiotic-antimycotic solution (Sigma, USA)]. The splenic lymphocyte density was adjusted to 1.5×10^6 cells/mL, and the viability of the freshly isolated cells was always above 95 % (trypan blue exclusion test). To monitor the various parameters in the present investigation, the control group (C group) was incubated for 12, 24, 36, 48, and 60 h without reagents. Although the cells were in their logarithmic growth phase, 10^{-6} mol/L Cd (Cd group), 10^{-7} mol/L Se (Se group), or the mixture of 10^{-7} mol/L Se and 10^{-6} mol/L Cd (Se+Cd group) were added, and the cells were incubated for 12, 24, 36, 48, and 60 h. The concentrations of Cd and Se used in this study were according to previous studies [27, 28].

The total RNA was isolated from the cells using TRIzol reagent according to the manufacturer's instructions (Invitrogen, USA). The RNA concentrations were determined using a GeneQuant 1300.

The reverse transcription reaction (40 μL) consisted of 10 μg of total RNA, 1 μL of M-MLV reverse transcription, 1 μL of RNase inhibitor, 4 μL of dNTP, 2 μL of Oligo dT, 4 μL of dithiothreitol, and 8 μL of 5× buffer. Reverse transcription was performed according to the manufacturer's instructions (Invitrogen, USA). The reverse transcription products (cDNA) were then stored at −20 °C for PCR.

To design the primers, we used chicken NF-κB, iNOS, COX-2, TNF-α, and PGE_2 mRNA GenBank sequences with accession numbers NM_205134, NM_204961, NM_001167718, NM_204267, and NM_00119483, respectively. Chicken β-actin (GenBank accession number L08165.1) as a housekeeping gene was used as an internal reference. The primers (Table 1) were designed using Prime

Table 1 Primers used for quantitative real-time PCR

Gene	Serial number	Primer (5′→3′)	Product size (bp)
β-Actin	L08165.1	Forward: CCGCTCTATGAAGGCTACGC Reverse: CTCTCGGCTGTGGTGGTGAA	128
NF-κB	NM_205134	Forward: TCAACGCAGGACCTAAAGACAT Reverse: GCAGATAGCCAAGTTCAGGATG	162
iNOS	NM_204961	Forward: CCTGGAGGTCCTGGAAGAGT Reverse: CCTGGGTTTCAGAAGTGGC	82
COX-2	NM_001167718	Forward: TGTCCTTTCACTGCTTTCCAT Reverse5: TTCCATTGCTGTGTTTGAGGT	84
TNF-α	NM_204267	Forward: GCCCTTCCTGTAACCAGATG Reverse: ACACGACAGCCAAGTCAACG	82
PGE_2	NM_001194983	Forward: GTTCCTGTCATTCGCCTTCTAC Reverse: CGCATCCTCTGGGTTAGCA	115

5 Software and were synthesized by Invitrogen Biotechnology Co., Ltd. in Shanghai, China.

Real-time quantitative PCR was used to detect the expression of NF-κB, iNOS, COX-2, TNF-α, and PGE_2 genes in the cells using SYBR Premix Ex Taq™ (Takara, China), and real-time PCR work was performed using an ABI PRISM 7500 real-time PCR system (Applied Biosystems). The program was 1 cycle at 95 °C for 30 s, then 40 cycles at 95 °C for 5 s, and finally at 60 °C for 34 s. The dissociation curves were analyzed using Dissociation Curve 1.0 Software (Applied Biosystems) for each PCR reaction to detect and eliminate possible primer-dimer and nonspecific amplification. The mRNA relative abundance was calculated according to the method of Pfaffl [29].

NO Level and iNOS Activity Assay

The NO content and iNOS activity in the chicken splenic lymphocytes were measured by a spectrophotometer (7230G, Shanghai Jinghua, Shanghai, China). The iNOS activities were spectrophotometrically measured at 530 nm using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) based on the oxidation of oxyhemoglobin to methemoglobin by NO. The data were expressed as units of iNOS activity per milligram of protein. The concentration of nitrite was measured to reflect the production of NO using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's protocol. In brief, the supernatant was mixed with the Griess reagent (1 % sulfanilamide, 0.1 % N-1-naphathylethylenediamine dihydrochloride, and 2.5 % phosphoric acid) at room temperature for 10 min. The nitrite products in the supernatants were determined by measuring the absorbance at 550 nm, using $NaNO_2$ as the standard curve. The results were expressed as nanomoles per milligram of protein.

Determination of Protein Content

Protein content was determined using the dye-binding method of Bradford [30]. Bovine serum albumin (BSA) was used to construct the standard curve.

Western Blot Analysis of NF-κB and COX-2

The chicken splenic lymphocytes of the C group, the Cd group, the Se group and the Se+Cd group were incubated for 12, 24, 36, 48, and 60 h. The cells were lysed in cell lysis solution, and then, the cells were centrifuged at 13,000×*g* for 5 min at 4 °C. The supernatants were stored at −80 °C until analysis by Western blot. The protein concentration was measured according to Bradford [30] using bovine serum albumin (BSA) as standard. Equal amounts of protein (80 μg/condition) were resolved in 15 % sodium dodecyl sulfate gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes. The proteins were transferred (200 mA for 60 min) to PVDF membranes using a Mini Trans-Blot Cell apparatus (Bio-Rad, Hercules, CA). The membranes were blocked with 5 % nonfat dry milk or BSA in PBST [10 mM Tris–HCl (pH 7.6), 100 mM NaCl, and 0.1 % Tween 20] overnight at 4 °C. The membranes were incubated for 1 h at 37 °C with the primary antibodies NF-κB and COX-2 and diluted to 1:100 and 1:200, respectively, in PBST+10 % nonfat-dried milk. After washing for four 5-min periods with PBST, the membranes were incubated for 1 h at 37 °C with peroxidase-conjugated secondary antibodies against rabbit IgG (1:1000, Santa Cruz, USA). After washing for four 5-min periods, the detection of bound antibodies was visualized by chemiluminescence using the ECL-plus reagent (GE Healthcare, Buckinghamshire, UK). The actin content was analyzed as a control using a rabbit polyclonal antibody (from Sigma).

Statistical Analysis

Statistical analyses were performed using SPSS for Windows (version 13; SPSS Inc., Chicago, IL, USA). When a significant value ($p<0.05$) was obtained according to one-way analysis of variance, further analyses were carried out. All data exhibited a normal distribution and passed equal variance testing. Differences between means were assessed using a Tukey's honestly significant difference test for post hoc multiple comparisons. The data are expressed as the mean±standard deviation. In addition, a 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.

Results

NO Levels and iNOS Activity in Chicken Splenic Lymphocytes

As shown in Fig. 1, the NO levels and iNOS activity significantly increased ($P<0.05$) in the chicken splenic lymphocytes (at 12, 24, 36, 48, and 60 h) for the Cd treatment group compared to the control groups. Cd/Se co-treatment decreased NO production and iNOS activities compared to the Cd groups, apart from NO production at 12 h. NO levels and NOS activity showed no significant ($P>0.05$) differences between the control and Se groups.

Effects of the mRNA Level of NF-κB, iNOS, COX-2, TNF-α, and PGE_2 in Chicken Splenic Lymphocytes

Pro-inflammatory cytokine levels were measured in the culture media by RT-PCR to determine the potential effects of Cd on the mRNA levels of NF-κB, iNOS, COX-2, TNF-α, and PGE2. The mRNA levels of NF-κB, iNOS, COX-2, TNF-α, and PGE_2 increased significantly ($P<0.05$ or $P<0.01$) (Fig. 2) in the chicken splenic lymphocytes of the Cd treatment groups compared with the corresponding control groups at 12, 24, 36, 48, and 60 h. Our results showed that in the Se+Cd group, the mRNA levels of iNOS, COX-2, TNF-α, and PGE2 were significantly reduced compared to the Cd group, but their values were not normally restored to the levels of the control group. The mRNA levels of these genes showed no significant differences between the control and Se groups, apart from iNOS and TNF-α at 60 h.

Effects of the Protein Expression of NF-κB and COX-2 in Chicken Splenic Lymphocytes

Western blot experiments showed the induction of NF-κB and COX-2 protein expression in the chicken splenic lymphocytes (Fig. 3). The expression of NF-κB and COX-2 protein increased significantly ($P<0.05$ or $P<0.01$) in the chicken splenic lymphocytes of the Cd treatment groups compared to the corresponding control groups at 12, 24, 36, 48, and 60 h. Our results showed that in the Se+Cd group, the protein expression levels of NF-κB and COX-2 were significantly decreased compared to the Cd group, apart from NF-κB at 12 h and COX-2 at 48 and 60 h, but these levels were not restored to the control levels. In addition, the protein expression levels of NF-κB and COX-2 showed no significant differences between the control and Se groups, apart from COX-2 at 60 h.

Chemometrics

Using PCA, the measured parameters were distinguished on ordination plots that corresponded to the first and second principal components (72.72 and 10.54 %, respectively) (Fig. 4). Furthermore, the observed correlations among the parameters were confirmed and quantified according to Spearman's test (Table 2). The result indicated that for all of the biomarkers, (a) the mRNA expressions of NF-κB, iNOS, COX-2, TNF-α,

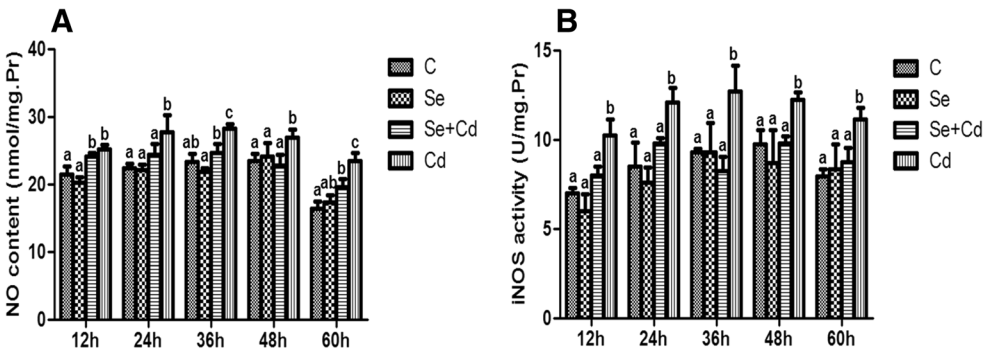

Fig. 1 NO production and iNOS activity in the chicken splenic lymphocytes. Effects of Se on Cd-induced changes on NO production and iNOS activity in the chicken splenic lymphocytes. **a** The effects of Se on Cd-induced changes on NO production. **b** The effects of Se on Cd-induced changes on iNOS activity. Significant differences: different letters indicate significant differences ($P<0.05$) between any two groups. Each value represents the mean±SD of five individuals

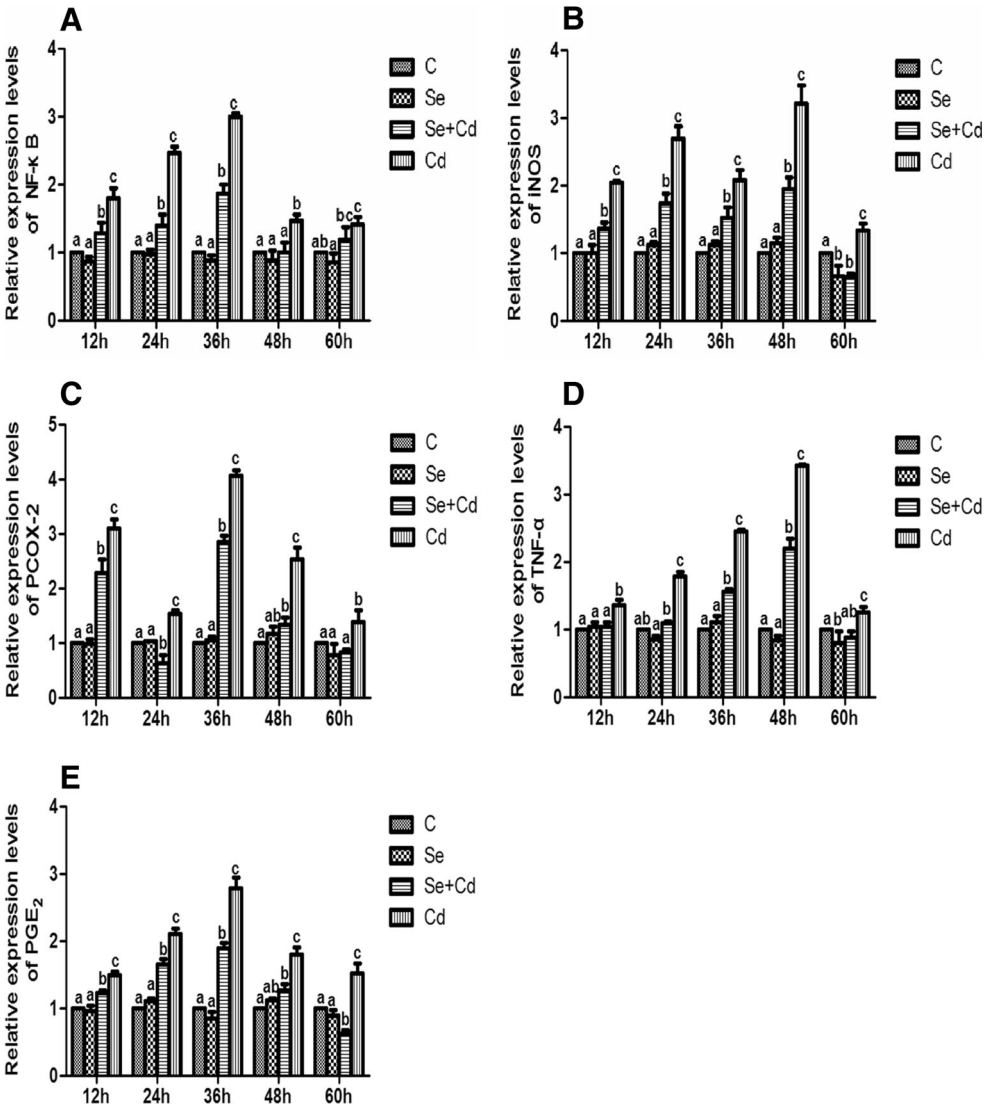

Fig. 2 The mRNA levels of NF-κB, iNOS, COX-2, TNF-α, and PGE_2 in chicken splenic lymphocytes. Effects of Se on Cd-induced changes in the mRNA levels of NF-κB, iNOS, COX-2, TNF-α, and PGE_2 in the chicken splenic lymphocytes. The relative mRNA levels from the C groups were used as reference values. The different letters indicate significant differences ($P<0.05$) between any two groups. Each value represents the mean±SD of five individuals

and PGE_2 were positively correlated with the protein expression of NF-κB and COX-2, and (b) the levels of inflammatory cytokines (NF-κB, iNOS, COX-2, TNF-α, and PGE_2) were positively correlated with NO production and iNOS activity. Moreover, the observed correlations among the parameters were confirmed and quantified using the Spearman's test (Table 2).

Discussion

Cd is regarded as an inflammation-related xenobiotic, as it induces complex inflammatory responses in several cell types. Several studies have documented the immunosuppressive effects of Cd on the immune system [31]. The immune system is one of the primary targets for Cd toxicity. Moreover, Cd accumulation in the body depends on the route, dose, and duration of exposure [21]. Our studies revealed the effects of Se against Cd toxicity in chicken splenic lymphocytes by ameliorating some selective inflammatory factors, as shown in Fig. 5. Taken together, our results demonstrated that Cd induces inflammation, as inflammatory molecules, NO production, and iNOS activity increased. The mRNA expression of the cytokines (NF-κB, iNOS, COX-2, TNF-α, and PGE_2) increased due to Cd exposure. Interestingly, our experimental results also revealed Se as an effective inhibitor of Cd-induced cytokines, such as NO content and iNOS activity, and of the expression levels of iNOS, TNF-α, and COX-2 via a blockade of NF-κB activation in the chicken splenic lymphocytes.

NO is an important signaling molecule, and low levels of NO regulate various physiological processes in the nervous, cardiovascular and immunological systems, but the overproduction of NO is involved in pesticide and heavy metal toxicity [16]. NO is a free radical and can be generated by iNOS, which is an enzyme expressed after cells are exposed to several noxious agents and can be induced by various cytokines

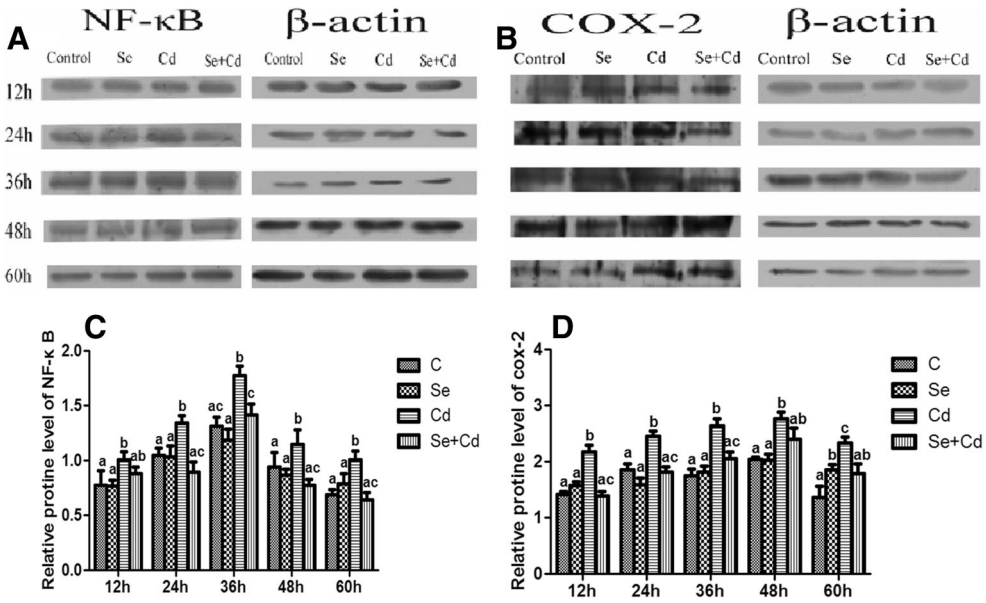

Fig. 3 The expression of NF-κB and COX-2 proteins in the chicken splenic lymphocytes. Effects of Se on Cd-induced changes in the expression of NF-κB and COX-2 proteins in the chicken splenic lymphocytes. β-Actin was used as a control. The relative expression levels from the C groups were used as reference values. The *different letters* indicate significant differences ($P<0.05$) between any two groups. Each value represents the mean±SD of five individuals

to generate large amounts of NO [32]. A previous study [33] found that chronic Cd exposure significantly induced NO production and the iNOS activity of macrophages in mice. Yang et al. found that Cd toxicity caused increased iNOS expression, NO overgeneration, and apoptosis in the ovaries of chickens [34]. Soyupek et al. [35] found that Cd poisoning increased NOS isoenzyme levels in the kidney and affected renal physiology in rats. Li et al. [24] correlated the toxic effects of Cd on the liver with an increase of oxidative stress, NO production and iNOS activity. In the present study, we detected a significant increase in iNOS gene expression, iNOS activity, and NO production in the lymphocytes of Cd-exposed chickens. We considered that Cd exposure conditions appeared to induce the release of many inflammatory mediators and to stimulate the immune cells to increase iNOS expression and NO production. At the same time, the overproduction of NO participated in Cd-induced immunotoxicity in the chicken splenic lymphocytes was observed.

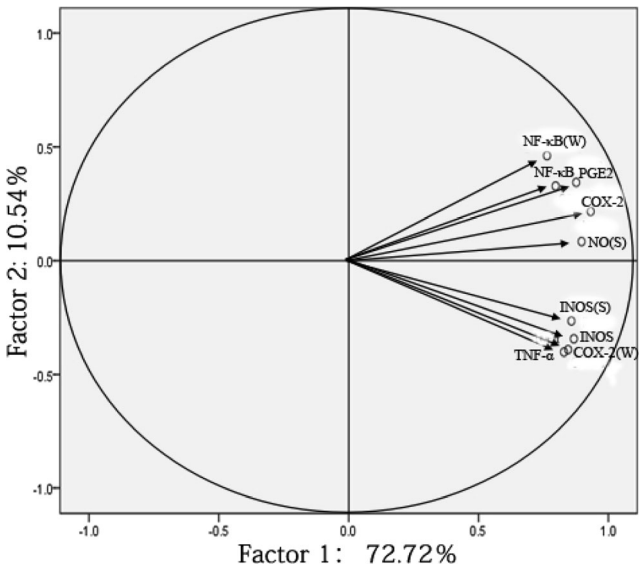

Fig. 4 Ordination diagram of PCA of the biochemical parameters measured in the chicken splenic lymphocytes after exposure to Se, Cd, or a mixture of the two for 12, 24, 36, 48, and 60 h. NF-κB, iNOS, COX-2, TNF-α, and PGE2 represent in the mRNA levels of NF-κB, iNOS, COX-2, TNF-α, and PGE_2; NF-κB(W) and COX-2(W) represent in the protein levels of NF-κB and COX-2; NO(S) and iNOS(S) represent in NO production and iNOS activity

NF-κB is a redox-sensitive transcription factor [36] and a major regulator of immune and stress responses. In a previous report, it was shown that Cd induced the expression of intercellular adhesion molecule-1 (ICAM-1) via NF-κB activation [37]. In addition to Cd, several metals exerted effects on the activation and activity of NF-κB; for example, manganese (Mn) appeared to increase the DNA binding activity of NF-κB [38]. It has been already shown that NF-κB can be activated by TNF-α, and then, the downstream cytokines of NF-κB, such as COX-2 and iNOS, are activated. The induction of COX-2 and iNOS can then produce PGEs and NO [39, 40]. As an important limiting enzyme, COX-2 is involved in the biosynthesis of inflammatory prostaglandins [41], which generate free oxygen radicals that can result in injury. Moreover, inflammatory reactions are facilitated when TNF-α interacts with NF-κB. Similar results were observed in our study, since the severity of the injury of the chicken splenic lymphocytes increased significantly with increasing TNF-α and exposure times. In the present study, NF-κB appeared as a critical factor influencing the expression of various cytokines, and increased expression levels of iNOS and COX-2 in the chicken splenic lymphocytes were observed as a

Table 2 Correlation coefficients amongst the parameters measured in the chicken splenic lymphocytes

	COX-2	iNOS	NF-κB	PGE_2	TNF-α	COX-2(W)	NF-κB(W)	NO(S)
iNOS	0.583**							
NF-κB	0.777**	0.636**						
PGE_2	0.766**	0.748**	0.922**					
TNF-α	0.622**	0.875**	0.544**	0.678**				
COX-2(W)	0.538**	0.762**	0.616**	0.696**	0.813**			
NF-κB(W)	0.659**	0.455*	0.747**	0.764**	0.468*	0.508*		
NO(S)	0.684**	0.817**	0.768**	0.841**	0.654**	0.651**	0.705**	
iNOS(S)	0.503*	0.744**	0.695**	0.713**	0.702**	0.887**	0.581**	0.736**

In addition, NF-κB, iNOS, COX-2, TNF-α, and PGE2 represent in the mRNA levels of NF-κB, iNOS, COX-2, TNF-α, and PGE2; NF-κB(W) and COX-2(W) represent in the protein levels of NF-κB, COX-2; NO(S) and iNOS(S) represent in the NO production and the iNOS activity

*$P<0.05$ according to Spearman's test; **$P<0.01$ according to Spearman's test

response to Cd. Similar results were also illustrated in the protein expression levels of NF-κB and COX-2. As inflammation could play a major role in chicken splenic lymphocyte damage produced due to exposure to Cd, it is clear that Cd induced COX-2 expression and, thereby, the release of PGE_2. Our results were consistent with a previous report by Hseu et al. who demonstrated that cellular transformation was associated with enhanced transcription of COX-2 and increased production of PGE_2 [32]. These data confirmed the inflammatory response in the immune cells and the pro-inflammatory cytokines that may be activated by Cd exposure. Taking into consideration the possible relationship between inflammatory responses and the overproduction of NO, we hypothesized that inflammatory factors may play a role in the lymphocyte injury induced by Cd.

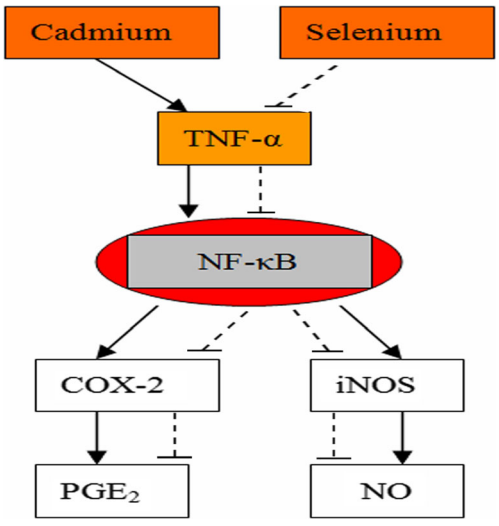

Fig. 5 The proposed mechanisms of the effect of Se on the Cd-induced inflammatory cascade. →, activation; ⊣, inhibition. *NF-κB* nuclear factor κappaB, *COX-2* cyclooxygenase-2, *iNOS* inducible nitric oxide synthase, TNF-α tumor necrosis factor-α, *PGE_2* prostaglandin E_2, *NO* nitric oxide

The immunoprotective effects of Se can be partially attributed to the properties of anti-inflammatory agents and antioxidants. The antioxidant effect leads to oxygen-free radical scavenging. Zhao et al. showed that Se can ameliorate Cd-induced oxidative stress or improve the efficacy of the antioxidant defense system in the immune organs of chickens [42]. Some studies have showed that Se is involved in the metabolism and regulation of inflammatory cytokines. Zhang et al. [43] illustrated that Se deficiency could induce iNOS activity and NO overproduction in the immune tissue of chickens. In addition, Se deficiency could result in pancreatic injury by influencing NO and selenoprotein function in the pancreas of chickens [44, 45]. Se supplementation is involved in protecting cells and tissues against damage caused by free radicals [46]. Recent research has demonstrated that Se could reduce the expression level of TNF-α in the case of cerebral ischemia–reperfusion injury in rats [47, 48]. At the same time, NF-κB is a major regulator of immune and stress responses, and the activation and inhibition of NF-κB are closely correlated with Se supply. Some studies have clearly indicated that Se attenuates the increase in cytokines expression, probably through NF-κB activity inhibition [32]. The administration of Se with Cd has protected the lymphocytes from Cd intoxication, as indicated by the significant restoration of cytokines levels. The protective effects of Se on the hematological changes in Cd-exposed rats could be attributed to modulator inflammatory cytokines or the redistribution of Cd in different organs [49]. Consistent with these reports, our study confirmed the inflammatory responses in the immune cells of chicken and that the expression level of pro-inflammatory cytokine-TNF-α may be inhibited by Se supplementation. Furthermore, Se acted on Cd-induced immunotoxicity via the inhibition of NF-κB, COX-2, iNOS, TNF-α, and PGE_2 expression in the chicken

splenic lymphocytes (Fig. 5). These results demonstrated that Se had a protective role against Cd-induced inflammation in the Se-treated group compared to the corresponding control group.

Conclusion

In summary, the increased levels of NF-κB, COX-2, iNOS, TNF-α, and PGE_2 further confirmed the relationship between inflammatory reactions and immune cells' exposure to Cd. Se attenuated the Cd-induced inflammatory reaction, which was mediated at least in part by a downregulation of cytokine expression via the suppression of NF-κB activation. Although further studies are required, we propose that Se decreases Cd immunotoxicity.

Acknowledgments This study was supported by the National Natural Science Foundation of China (Grant No. 31472161).

Ethical Standards All chicken experiments were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University under the approved protocol number SRM-06.

Conflicts of Interest The authors declare that there are no conflicts of interest.

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