Biol Trace Elem Res (2016) 170:237–244
DOI 10.1007/s12011-015-0442-0

Effects of Molybdenum or/and Cadmium on mRNA Expression Levels of Inflammatory Cytokines and HSPs in Duck Spleens

Huabin Cao[1] · Mengmeng Zhang[1] · Bing Xia[1] · Jin Xiong[1] · Yibo Zong[1] · Guoliang Hu[1] · Caiying Zhang[1]

Received: 17 June 2015 / Accepted: 7 July 2015 / Published online: 24 July 2015
© Springer Science+Business Media New York 2015

Abstract Cadmium (Cd) and high dietary intake of molybdenum (Mo) can cause multiple-organ injury in animals, but the co-induced toxicity of Mo and Cd to spleen in ducks is not well understood. The aim of this study was to investigate the co-induced effects of Mo and Cd on the mRNA expression levels of inflammatory cytokines and heat shock proteins (HSPs) in duck spleens. Two hundred forty healthy 11-day-old ducks were randomly divided into six groups and treated with a commercial diet containing Mo or/and Cd. After being treated with Mo or/and Cd for 30, 60, 90, and 120 days, the mRNA expression levels of nuclear factor-kappa B (NF-κB), tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2), HSP60, HSP70, and HSP90 were examined in duck spleens. Histopathology was examined in duck spleens at 120 days. The results indicated that the mRNA expression levels of HSPs were significantly upregulated in the co-induced groups ($P < 0.01$), while these decreased in the high dietary intake of Mo combined with Cd group at 120 days. Exposure to Mo or/and Cd upregulated the mRNA expression levels of NF-κB, COX-2, and TNF-α in the combination groups ($P < 0.01$). Furthermore, severe congestion, bleeding, splenic corpuscle structure fuzzy, wall thickness of sheath artery thickening, and oxyhematin were observed in the spleens of combination groups. Meanwhile, the organizational structure damage of the combined groups was more severe than that of the other groups. These results suggested that exposure to Mo or/and Cd might lead to tissue damage, and high expression of HSPs and inflammatory cytokines may play a role in the resistance of spleen toxicity induced by Mo or/and Cd. Interaction of Mo and Cd may have a synergistic effect on spleen toxicity.

Keywords Molybdenum · Cadmium · Spleen · Duck · Inflammatory cytokines · Heat shock proteins

Introduction

Molybdenum (Mo) is an important trace element in the nutrition of animals, plants, and microorganisms [1], which is distributed throughout the environment. Mo has been used widely for industrial stainless steel, mining, cast iron, fertilizer manufacture, and agricultural activities [2]. High dietary intake of Mo can cause multiple-organ injury in animals. The toxicity of molybdenum compounds has been studied intensively in animals [3]. It has been reported that molybdenosis resulted in depigmentation of the skin in cows and goats, defective hair structure in sheep, and testicular degeneration in goats [4]. High dietary intake of Mo induced oxidative stress as well as decreased antioxidant enzymatic activities and histopathological changes in rabbit testes [5].

Cadmium (Cd) is one of the most important heavy metal toxicants, and its environmental concentration is increased due to its extensive utilization in modern industries. It is now well

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

Huabin Cao, Mengmeng Zhang, and Bing Xia are the first authors.

✉ Guoliang Hu
hgljx@163.com

✉ Caiying Zhang
zhangcaiying0916@163.com

[1] Institute of Animal Population Health, College of Animal Science and Technology, Jiangxi Agricultural University, No. 1101 Zhimin Avenue, Economic and Technological Development District, Nanchang 330045, Jiangxi, People's Republic of China

accepted that cadmium can accumulate in many organs and adversely decrease the functions of these organs including the spleen, lung, kidney, liver, and urinary bladder [6]. Moreover, high Cd contamination in drinking water induced splenocyte proliferation in rats [7], and Cd intoxication induced cell apoptosis and reduced cell antioxidant capacity [8]. Recent studies have demonstrated that spleen is more sensitive to cadmium toxicity, and it is the target organ in cadmium toxicity [9–11].

It is well known that improper mining and industrialization could lead to an increase in concentration of some heavy metal elements in soil, water, and air. They can also be absorbed by aquatic and terrestrial organisms and consequently cause acute/chronic toxicity in animals. Southern Jiangxi Province is rich in mineral resources and contains large amounts of tungsten ore. In the mining process of tungsten ore, Mo and Cd in the tailing usually pollute water, land, vegetation, and waterfowl. The spleen is one of the major immune organs, sensitive to stress, and has a protective effect on the body. Therefore, this in vivo study was conducted with the core objective to understand the effects of Mo or/and Cd on heat shock proteins (HSPs), inflammatory cytokine-related gene expression, and histopathological changes in duck spleens.

Materials and Methods

Animals and Treatments

All animal care and experimental procedures were approved by the institutional ethics committee of Jiangxi Agricultural University. Hexaammonium molybdate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$) and cadmium sulfate ($3CdSO_4\cdot 8H_2O$) were used as sources of Mo and Cd, respectively. Two hundred forty healthy 11-day-old ducks were randomly divided into six groups ($n = 40$ per group). A duck model of excessive exposure to Mo or/and Cd was developed as described in our previous publication [12]. Briefly, ducks in each group were fed with basal diet with different concentrations of Mo or/and Cd: control group (0 mg/kg Mo, 0 mg/kg Cd), low dietary intake of Mo group (LMo group, 15 mg/kg Mo), high dietary intake of Mo group (HMo group, 100 mg/kg Mo), Cd group (4 mg/kg Cd), LMo + Cd group (15 mg/kg Mo, 4 mg/kg Cd), and HMo + Cd group (100 mg/kg Mo, 4 mg/kg Cd). The basal diet was formulated according to the National Research Council (NRC) (1994). Ducklings were fed with duckling basal diet and duck basal diet before and after 21 days old, respectively. The feeding experiment lasted for 120 days, and ducks were given free access to standard food and water. The ducks were handled and treated in accordance with the strict guiding principles of the National Institution of Health for experimental care and use of animals. Contents of Mo, Cd, Copper (Cu), Zinc (Zn), Iron (Fe), and selenium (Se) in the basal diet and water are shown in Table 1.

Sample Collection

Spleen tissues were removed immediately from random selection of ten ducks from each group after they were sacrificed with an overdose intravenous injection of sodium pentobarbital (Nembutal, Abbot Labs, IL, USA, 100 mg/kg) on days 30, 60, 90, and 120. The samples were immediately frozen by liquid nitrogen and then stored at −80 °C for RNA isolation. In addition, part of the spleen specimen at 120 days was fixed in formalin for the production of pathological sections.

Determination of Trace Elements

The trace elements including Mo, Cd, Mo, Cd, Cu, Fe, Zn, and Se in the water and feed were measured using a Shimadzu AA 680 flame atomic absorption spectrophotometer (Shimadzu, Japan).

RNA Isolation and Primer Designing

Total RNA was isolated from spleen tissue samples using TRIzol reagent according to the manufacturer's instructions (TaKaRa, Dalian, China). The concentration of the total RNA was determined using Thermo NanoDrop 2000 (Thermo Fisher Scientific Inc., Waltham, USA). First-strand complementary DNA (cDNA) synthesis was carried out with TaKaRa® RT-PCR Kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. The reverse transcription reaction (20 μL) was conducted in a mixture containing 2 μL of 5× DNA Eraser Buffer, 1 μL of gDNA Eraser, 1 μL of total RNA, and 6 μL of RNase-free dH_2O and was then incubated for 2 min in a 42 °C environment. Next, 4 μL of 5× Prime Script Buffer 2, 1 μL of Prime Script RT Enzyme Mix I, 1 μL of RT Primer Mix, and 4 μL of RNase-free dH_2O were added to the reaction solution, and the reaction was run at 37 °C for 15 min, 85 °C for 5 s, and 4 °C for 10 min. Primers for the amplification of genes tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2), nuclear factor-kappa B (NF-κB), HSP60, HSP70, HSP90, and β-actin were designed using Primer Premier Software (PREMIER Biosoft International, CA, USA). The primer sequences and GenBank accession numbers are shown in Table 2. Designed primers were optimized prior to quantification experiments using polymerase chain reaction. For these genes, the expected sizes of the products were confirmed by gel electrophoresis on 2 % agarose gel.

Real-Time Quantitative PCR

Gene expression levels were assessed by real-time quantitative polymerase chain reaction (RT-qPCR). The PCR cycles's condition was as follows: denaturation at 95 °C for 10 min, 40 cycles at 95 °C for 15 s, and 60 °C for 60 s and extension at

Table 1 Contents of Mo, Cd, Cu, Zn, Fe, and Se in the basal diet and water

Item	Contents of trace elements (μg g^{-1})					
	Mo	Cd	Cu	Zn	Fe	Se
Deionized water	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Tap water	0.0104	0.0075	0.0211	0.1520	0.1880	0.0151
Duckling feed	4.1510	0.2471	191.3511	210.7144	747.8350	1.0483
Duck feed	4.7290	0.4762	109.3302	189.8741	709.0814	1.0520

95 °C for 15 s. At the end of PCR reactions, melt curve analyses were performed for all genes. All reactions were carried out using the Light Cycler 96 real-time PCR machine (Roche Applied Science, Harbin, China). Relative expression was calculated based on qPCR efficiency (E) and threshold cycle difference (delta delta CT) of a treated versus control groups for both target and reference genes according to the formula:

$$\text{Ratio} = \left(E_{\text{target}}\right)\text{delta Ct}_{\text{target}}(\text{control}-\text{sample}) \Big/ \left(E_{\text{ref}}\right)\bullet\text{delta Ct}_{\text{ref}}(\text{control}-\text{sample})$$

All target gene transcriptions were expressed as n-fold difference relative to the calibrator. For each gene, standard curves were created using threefold dilution of cDNA and used to calculate the individual real-time PCR efficiencies ($E = 10[-1/\text{slope}]$). The internal reference (β-actin) genes were used as an internal control for normalization of the results.

Histopathologic Examination

The spleen tissue specimens at 120 days were fixed in formalin and routinely processed in paraffin. Thin sections (5~8 μm) of each tissue were sliced from each block and mounted on glass. Slides were stained with hematoxylin and eosin (H&E). Afterward, pathological sections were observed using an optical microscope and photographs were taken [13].

Statistical Analysis

Statistical analysis of all data was performed by using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.01 (GraphPad Inc., La Jolla, CA, USA). Differences between means were assessed by using one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. All data showed a normal distribution and passed equal variance testing. Data were expressed as mean ± SEM.

Results

The mRNA Expression of HSP60, HSP70, and HSP90

As shown in Fig. 1, HSP60 expression in the spleen was significantly increased ($P < 0.01$) in the LMo + Cd and HMo + Cd groups compared to other groups on days 30, 60,

Table 2 Gene special primers used in this study

Gene name	Accession number	Primer sequences (5′ to 3′)
HSP60	JQ669386.1	Forward: AGCCAAAGGGCAGAAATG
		Reverse: TACAGCAACAACCTGAAGACC
HSP70	KC706508.1	Forward: CATGAAGCAGACGGTGGAG
		Reverse: GATCGAGCCAAGAGATCACC
HSP90	KF130900.1	Forward: CCTTCAGCTGCTGCACACA
		Reverse: CGCATCTGGTGATGAAATGGT
TNF-α	EU375296.1	Forward: ACAGGACAGCCTATGCCAAC
		Reverse: ACAGGAAGGGCAACACATCT
COX-2	NM_001167718.1	Forward: TCCACCAACAGTGAAGGACA
		Reverse: GGACCAAGCCAAACACCTC
NF-κB	NM_205134	Forward: TCAACGCAGGACCTAAAGACAT
		Reverse: GCAGATAGCCAAGTTCAGGATG
β-Actin	EF667345.1	Forward: ATGTCGCCCTGGATTTCG
		Reverse: CACAGGACTCCATACCCAAGAAT

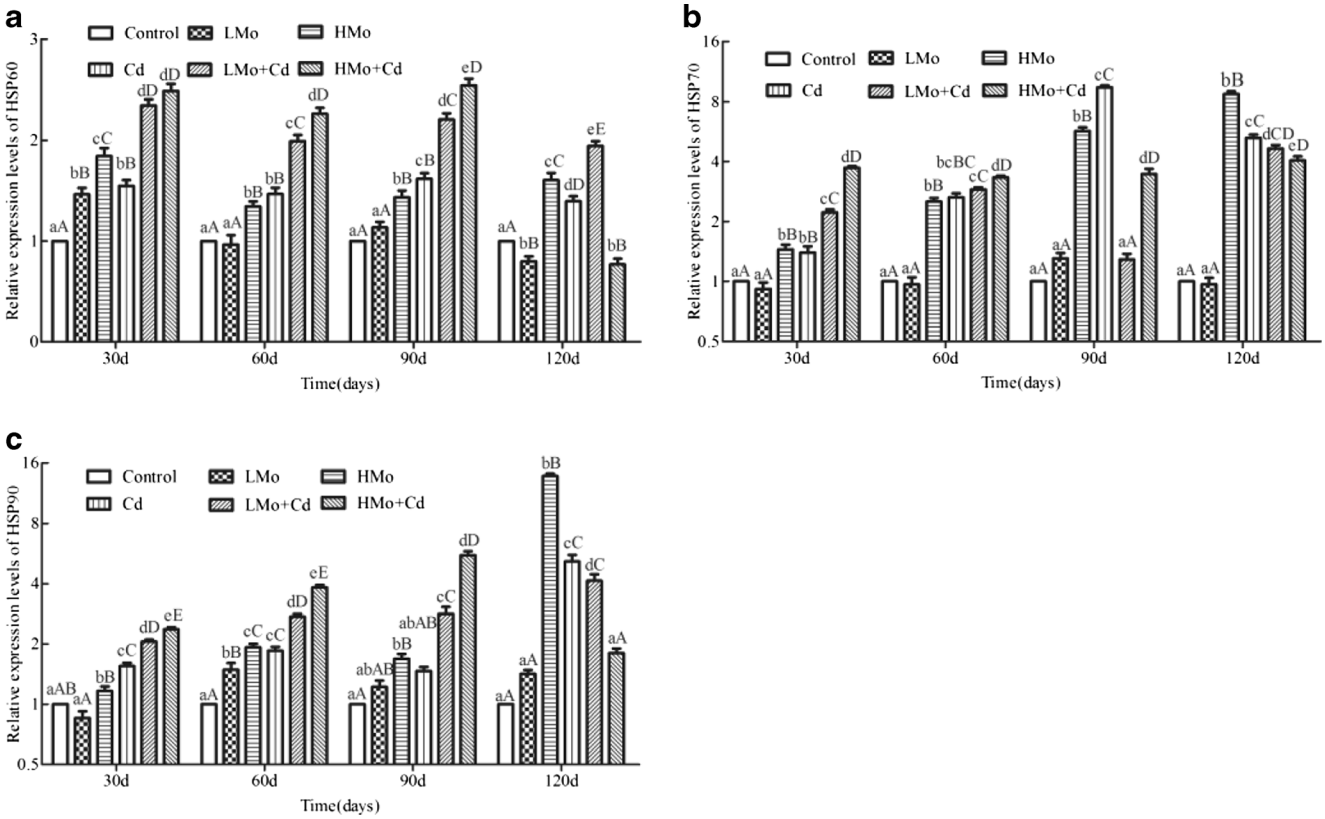

Fig. 1 Effects of Mo- or/and Cd-induced changes in the mRNA levels of HSPs. **a**–**c** The effects of Mo- or/and Cd-induced changes in the mRNA levels of HSP60, HSP70, and HSP90, respectively. Statistically significant differences: *means with different lowercase letters* are significantly different between groups ($P < 0.05$), *means with different uppercase letters* are highly significantly different between groups ($P < 0.01$), and *means with common lowercase or uppercase letters* are not significantly different between groups ($P > 0.05$). Each value represents the mean ± SEM

and 90. Additionally, HSP60 expression was significantly higher ($P < 0.01$) in the HMo + Cd group than in the LMo + Cd group on days 60 and 90. At 120 days, the mRNA expression level of HSP60 was significantly decreased ($P < 0.01$) in the HMo + Cd group compared to other groups except the LMo group (Fig. 1a). The mRNA expression level of HSP70 was highly significantly ($P < 0.01$) increased in the HMo + Cd group in comparison with the control group on days 30, 60, 90, and 120. HSP70 expression in the HMo + Cd group was markedly higher ($P < 0.01$) than that in the LMo + Cd group on days 30, 60, and 90. Moreover, the mRNA expression level of HSP70 was significantly decreased in the LMo + Cd and HMo + Cd groups compared with the HMo and Cd groups on days 90 and 120 (Fig. 1b). The mRNA expression level of HSP90 was significantly upregulated ($P < 0.01$) in the LMo + Cd and HMo + Cd groups compared to the other four groups on days 30, 60, and 90. Notably, HSP90 expression in the LMo + Cd group was significantly increased ($P < 0.01$) compared to that in the control and LMo groups, but HSP90 expression in the HMo + Cd group was significantly decreased ($P < 0.01$) compared to that in the HMo, Cd, and LMo + Cd groups (Fig. 1c).

The mRNA Expression of NF-κB, COX-2, and TNF-α

As shown in Fig. 2, the mRNA expression levels of TNF-α, COX-2, and NF-κB were upregulated in all the five treatment groups compared with the control group at all time points. TNF-α expression was significantly increased ($P < 0.01$) in the LMo + Cd group and HMo + Cd group on days 30, 60, 90, and 120 compared to the control group. Additionally, the expression of TNF-α was highly significantly increased ($P < 0.01$) in the HMo + Cd group in comparison with the other five groups on days 30, 60, 90, and 120 (Fig. 2a). The mRNA expression level of NF-κB was significantly upregulated ($P < 0.01$) in the LMo + Cd and HMo + Cd groups compared to the LMo, HMo, and Cd groups on days 90 and 120. NF-κB expression was highly upregulated ($P < 0.01$) in the HMo + Cd group than in the LMo + Cd group. However, there was no significant difference between ($P > 0.05$) the LMo + Cd group and the HMo + Cd group on days 30, 60, and 90 (Fig. 2b). The mRNA expression level of COX-2 was significantly increased ($P < 0.01$) in the five treatment groups compared with the control group at all time points. NF-κB expression was observed to be significantly increased ($P < 0.01$) in the

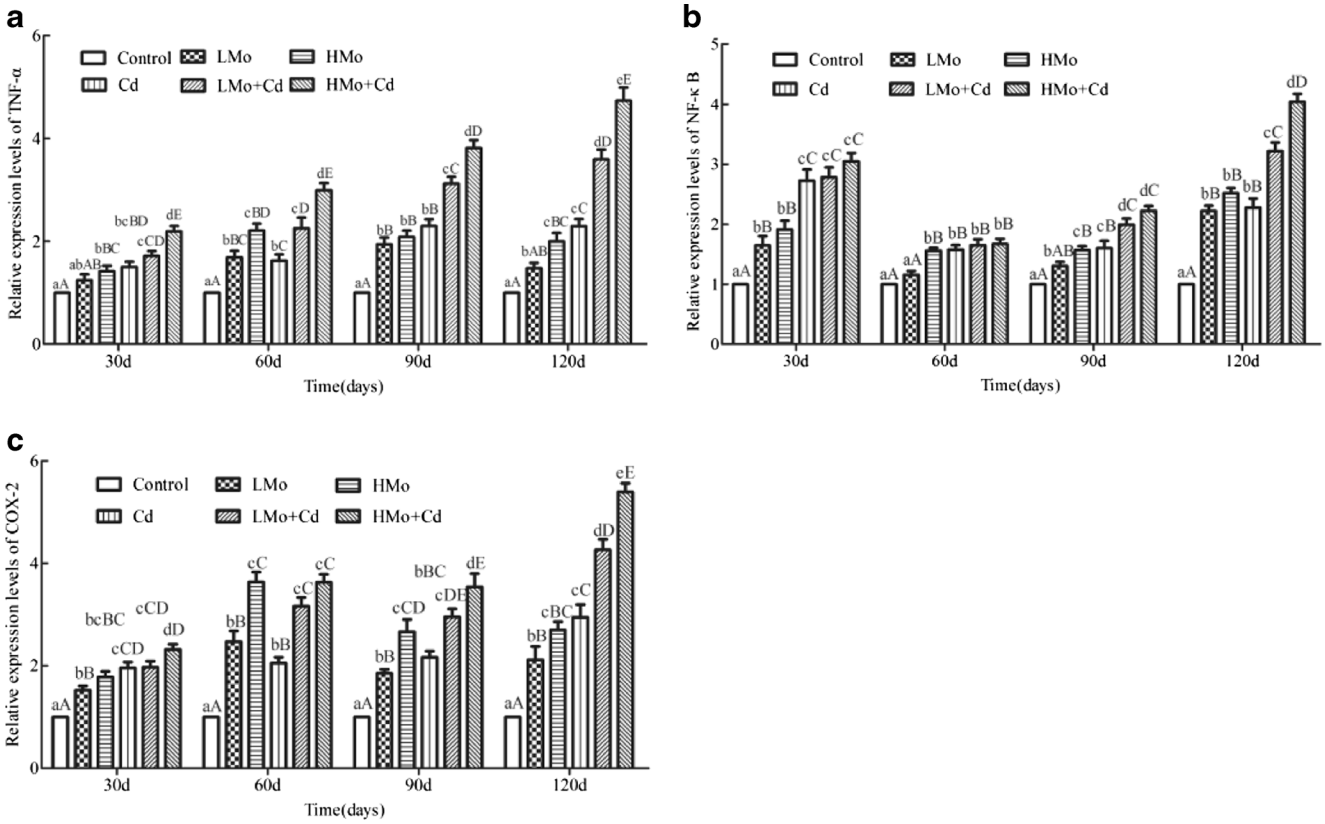

Fig. 2 Effects of Mo or/and Cd on the mRNA expression levels of NF-κB, COX-2, and TNF-α in duck spleens. **a**–**c** The effects of Mo- or/and Cd-induced changes in the mRNA levels of NF-κB, COX-2, and TNF-α, respectively

LMo + Cd and HMo + Cd groups compared to the LMo, HMo, and Cd groups on days 90 and 120. NF-κB mRNA expression level in the HMo + Cd group was highly up-regulated compared to that in the LMo + Cd group on days 90 and 120 (Fig. 2c).

Histopathology of the Spleen

The histopathological changes in the spleens at 120 days are shown in Fig. 3. The spleens from untreated ducks showed normal morphology including normal splenic corpuscle, normal wall thickness of sheath artery, white pulp, and red pulp obviously (Fig. 3a). A histological lesion showing a small amount of bleeding was noted in the LMo group (Fig. 3b). Congestion and bleeding were observed in the white pulp and splenic corpuscle structure fuzzy in the HMo and Cd groups (Fig. 3c, d). Additionally, treatment with Cd produced a thickened sheath artery wall (Fig. 3d). In the LMo + Cd and HMo + Cd groups, morphology of the spleens showed unclear red and white pulp boundaries, unrecognizable splenic corpuscle, and thickened sheath artery wall (Fig. 3e, f). In addition, oxyhematin was also observed in the HMo + Cd group (Fig. 3f).

Discussion

In the living environment, animals are often exposed to stress conditions such as oxidative stress, inflammation, heavy metals, or other toxic compounds [14]. When living organisms are exposed to various stress conditions, a group of anti-stress proteins known as heat shock proteins (HSPs) and inflammatory cytokines will be rapidly synthesized [15]. Metal pollution is a tremendous problem faced by the local government in southern Jiangxi Province.Molybdenum and cadmium are the main pollutants in the tailings in the process of mining, which pose hazardously threats to animals and public health.

Heat shock proteins are molecular chaperones that are involved in many normal cellular processes and stress responses [16]. HSPs represent a highly conserved class of cytoprotective proteins specifically induced at the cellular level in response to one of several environmental stressors and protect cells against environmental stress [17–19]. Some metals as a source of stress have previously been shown to increase HSP levels in various cell types and appear to mediate toxicity through a common oxidative stress mechanism [20]. Eunsook Lee et al. reported an excess of manganese-induced oxidative stress and inflammation [21]. Our previous

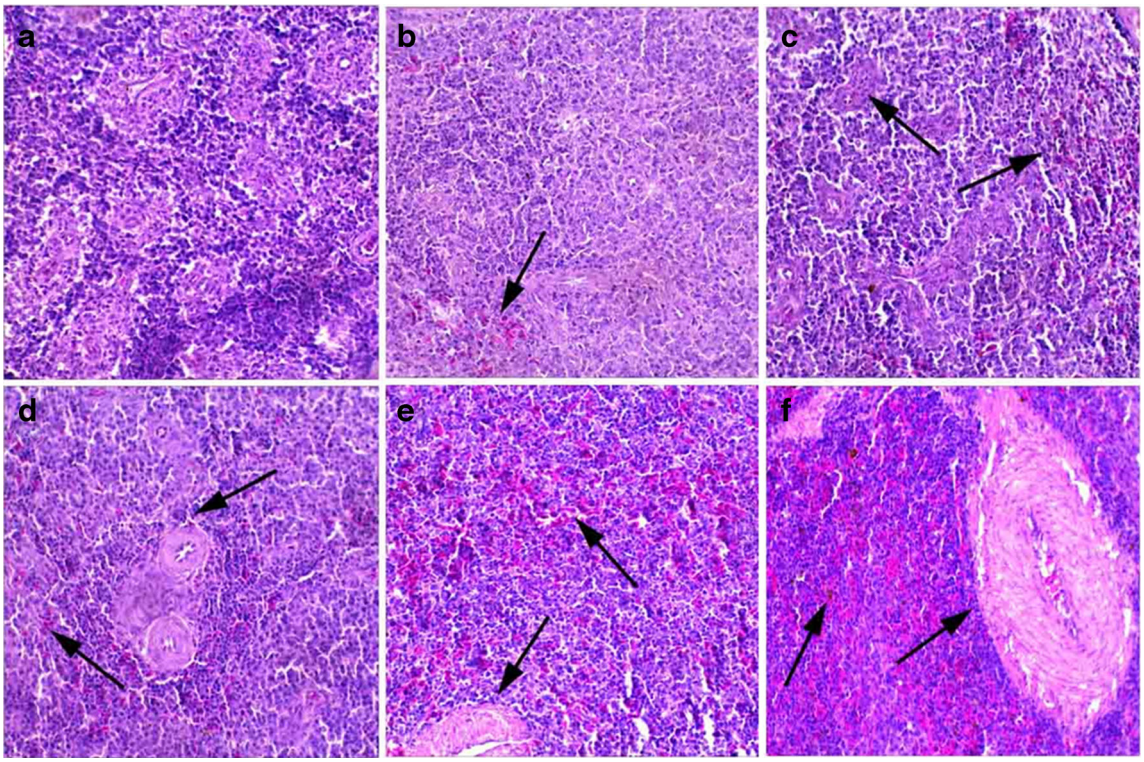

Fig. 3 Pathological observation results (hematoxylin and eosin staining, ×400). **a** Control group, **b** LMo group, **c** HMo group, **d** Cd group, **e** LMo + Cd group, and **f** HMo + Cd group. In **b**, the *arrowhead* means a small amount of bleeding in the spleen. In **c**, the *left arrowhead* means splenic corpuscle structure fuzzy and the *right arrowhead* means obvious bleeding in the white pulp. In **d**, the *left arrowhead* means obvious bleeding and the *right arrowhead* means thickened sheath artery wall. In **e**, the *left arrowhead* means thickened sheath artery wall and the *right arrowhead* means obvious bleeding. In **f**, the *left arrowhead* means oxyhematin appeared and the *right arrowhead* means thickened sheath artery wall

study has shown that the mRNA expression levels of HSPs in combination groups of Mo and Cd were upregulated; pathological changes in kidney tissue were obvious and the Mo + Cd groups were much more toxic than a single poisoning group [12]. In the present work, mRNA expression of HSPs was upregulated in the experimental groups while the HSP expression was decreased in the HMo + Cd group at 120 days. These results were consistent with Chen X and Ferencz A's study [22, 23]. Heat shock factors (HSFs) mediated transcription of HSPs under stress conditions. In the normal state, HSFs were in an inactive state in the cytoplasm of the monomer. When confronted with stress, HSF monomers and heat shock element (HSE) sequences bind and activate the transcription of HSP gene transregulatory factors and thus control the expression of HSPs [24]. By this way, the accumulated synthesis of HSPs caused HSPs and HSFs to combine to form a feedback regulation to inhibit transcription of HSP genes [25]. The spleen is one of the immune organs and sensitive to stress or stimuli coming from the environment. Exposure to Mo or/and Cd may break the balance of the immune system and reduce the immune organ function. In this study, the histopathological changes in duck spleens, incurred by cytotoxic effects of Mo or/and Cd, revealed the disappearance of certain red pulp and white pulp boundaries, congestion of the splenic pulp, hemorrhage, and appearance of oxyhematin. Additionally, the combination groups were seriously injured than the single groups. These results implied that molybdenum and cadmium may have a synergistic effect on the histology structure in duck spleens, and we postulated that tissue damage might be one of the mechanisms and the combination of molybdenum and cadmium will lead to much more reduction of immune function than molybdenum or cadmium independently.

Inflammation is an important indicator of animal tissue injury caused by noxious physical or chemical stimuli, and is a key component of multiple pathologies. TNF-α is a key proinflammatory cytokine regulating inflammation and plays roles in both homeostasis and disease pathophysiology [26]. NF-κB plays an important role in inflammatory processes and is closely related to the expressions of COX-2, iNOS, and TNF-α [27, 28]. COX-2 is an inducible enzyme that is activated by proinflammatory cytokines, tumor promoters, oncogenes, and growth factors [29]. Park MH et al. found that TNF-α can regulate the expression level of NF-κB by activating the classical pathway of NF-κB. Activated NF-κB also can be a feedback regulatory mechanism in the expression

of inflammatory cytokines, including iNOS, IL-6, COX-2, and chemokines [30]. Meanwhile, it was confirmed that HSP70 elicited a rapid intracellular calcium flux, activated NF-κB, and upregulated the expression of proinflammatory cytokines and TNF-α in human monocytes [31]. In the present study, ducks were used as a model to understand the mechanisms of Mo- or/and Cd-induced inflammatory response. The results presented that expression levels of COX-2, NF-κB, and TNF-α were all upregulated in all treated groups, indicating that inflammatory cytokines were overexpressed in the spleen which were in agreement with Du and Chen's findings about metal-induced excessive expression of inflammatory cytokines [32, 33]. Notably, the expression levels were significantly higher in the Mo combined with Cd groups than in the single groups in the later period of the experiment. The results revealed that mRNA expression of NF-κB, COX-2, and TNF-α increased along with Mo or/and Cd dietary supplement.

Conclusion

This study showed that Mo or/and Cd upregulated mRNA expression of NF-κB, COX-2, TNF-α, and HSPs. Morphological observation showed that histological changes were obvious. The results speculated that Mo and Cd may have a synergistic effect on spleen toxicity and may induce an immune system response to stress via two ways: heat shock proteins and inflammatory response through the NF-κB-COX-2 signaling pathway. Due to the molecular mechanisms underlying the effects exerted by Mo and Cd on relative gene expression unclear, the mechanism needs to be further investigated.

Acknowledgments This work was supported by the National Natural Science Foundation of China (No. 31260625, Beijing, People's Republic of China) and the Training Plan for Young Scientists of Jiangxi Province (No. 2014BCB23040, Nanchang, People's Republic of China). All authors thank all members of the team for their help in the experimental process in clinical veterinary medicine laboratory in the College of Animal Science and Technology, Jiangxi Agricultural University.

Conflict of Interest The authors declare that they have no competing interests.

References

1. McEwan AG, Ridge JP, McDevitt CA, Hugenholtz P (2002) The DMSO reductase family of microbial molybdenum enzymes; molecular properties and role in the dissimilatory reduction of toxic elements. Geomicrobiol J 19:3–21
2. Kumchai J, Huang JZ, Lee CY, Chen FC, Chin SW (2013) Proline partially overcomes excess molybdenum toxicity in cabbage seedlings grown in vitro. Genet Mol Res 12:5589–5601. doi:10.4238/2013.November.18.8
3. Othman AR, Bakar NA, Halmi MI, Johari WL, Ahmad SA, Jirangon H, Syed MA, Shukor MY (2013) Kinetics of molybdenum reduction to molybdenum blue by Bacillus sp. strain A.rzi. Biomed Res Int. doi:10.1155/2013/371058
4. Aupperle H, Schoon HA, Frank A (2001) Experimental copper deficiency, chromium deficiency and additional molybdenum supplementation in goats—pathological findings. Acta Vet Scand 42: 311–321
5. Bersenyi A, Berta E, Kadar I, Glavits R, Szilagyi M, Fekete SG (2008) Effects of high dietary molybdenum in rabbits. Acta Vet Hung 56:41–55. doi:10.1556/AVet.56.2008.1.5
6. Bulat ZP, Djukić-Cosić D, Malicević Z, Bulat P, Matović V (2008) Zinc or magnesium supplementation modulates Cd intoxication in blood, kidney, spleen, and bone of rabbits. Biol Trace Elem Res 124:110–117. doi:10.1007/s12011-008-8128-5
7. Suntararuks S, Yoopan N, Rangkadilok N, Worasuttayangkurn L, Nookabkaew S, Satayavivad J (2008) Immunomodulatory effects of cadmium and Gynostemma pentaphyllum herbal tea on rat splenocyte proliferation. J Agric Food Chem 56:9305–9311. doi: 10.1021/jf801062z
8. Risso-de Faverney C, Orsini N, de Sousa G, Rahmani R (2004) Cadmium-induced apoptosis through the mitochondrial pathway in rainbow trout hepatocytes: involvement of oxidative stress. Aquat Toxicol 69:247–258
9. Pathak N, Khandelwal S (2007) Role of oxidative stress and apoptosis in cadmium induced thymic atrophy and splenomegaly in mice. Toxicol Lett 169:95–108
10. Holaskova I, Elliott M, Hanson ML, Schafer R, Barnett JB (2012) Prenatal cadmium exposure produces persistent changes to thymus and spleen cell phenotypic repertoire as well as the acquired immune response. Toxicol Appl Pharmacol 265:181–189. doi:10.1016/j.taap.2012.10.009
11. Suresh N (2009) Effect of cadmium chloride on liver, spleen and kidney melano macrophage centres in Tilapia mossambica. J Environ Biol 30:505–508
12. Xia B, Cao H, Luo J, Liu P, Guo X, Hu G, Zhang C (2015) The co-induced effects of molybdenum and cadmium on antioxidants and heat shock proteins in duck kidneys. Biol Trace Elem Res. doi:10.1007/s12011-015-0348-x
13. Horiguchi H, Oguma E, Kayama F (2011) Cadmium induces anemia through interdependent progress of hemolysis, body iron accumulation, and insufficient erythropoietin production in rats. Toxicol Sci 122:198–210. doi:10.1093/toxsci/kfr100
14. Bozaykut P, Ozer NK, Karademir B (2014) Regulation of protein turnover by heat shock proteins. Free Radic Biol Med 77:195–209. doi:10.1016/j.freeradbiomed.2014.08.012
15. Al-Aqil A, Zulkifli I (2009) Changes in heat shock protein 70 expression and blood characteristics in transported broiler chickens as affected by housing and early age feed restriction. Poult Sci 88: 1358–1364. doi:10.3382/ps.2008-00554
16. Zhang J, Liu B, Li J, Zhang L, Wang Y, Zheng H, Lu M, Chen J (2015) Hsf and Hsp gene families in Populus: genome-wide identification, organization and correlated expression during development and in stress responses. BMC Genomics. doi:10.1186/s12864-015-1398-3
17. Purandhar K, Jena PK, Prajapati B, Rajput P, Seshadri S (2014) Understanding the role of heat shock protein isoforms in male fertility, aging and apoptosis. World J Mens Health 32:123–132. doi: 10.5534/wjmh.2014.32.3.123
18. Ou JR, Tan MS, Xie AM, Yu JT, Tan L (2014) Heat shock protein 90 in Alzheimer's disease. Biomed Res Int. doi:10.1155/2014/796869
19. Liu HH, He JY, Chi CF, Shao J (2014) Differential HSP70 expression in Mytilus coruscus under various stressors. Gene 543:166–173. doi:10.1016/j.gene.2014.04.008

20. Jimi S, Uchiyama M, Takaki AYA, Suzumiya J, Hara S (2004) Mechanisms of cell death induced by cadmium and arsenic. Ann N Y Acad Sci 1011:325–331
21. Lee E, Yin Z, Sidoryk-Wegrzynowicz M, Jiang H, Aschner M (2012) 15-Deoxy-Δ12,14-prostaglandin J_2 modulates manganese-induced activation of the NF-κB, Nrf2, and PI3K pathways in astrocytes. Free Radic Biol Med 52:1067–1074. doi:10.1016/j.freeradbiomed.2011.12.016
22. Ferencz A, Juhasz R, Butnariu M, Deer AK, Varga IS, Nemcsok J (2012) Expression analysis of heat shock genes in the skin, spleen and blood of common carp (Cyprinus carpio) after cadmium exposure and hypothermia. Acta Biol Hung 63:15–25. doi:10.1556/ABiol.63.2012.1.2
23. Chen X, Zhu YH, Cheng XY, Zhang ZW, Xu SW (2012) The protection of selenium against cadmium-induced cytotoxicity via the heat shock protein pathway in chicken splenic lymphocytes. Molecules 17:14565–14572. doi:10.3390/molecules171214565
24. Voellmy R (2005) Dominant-positive and dominant-negative heat shock factors. Methods 35:199–207
25. Zhu J, Katz RJ, Quyyumi AA, Canos DA, Rott D, Csako G, Zalles-Ganley A, Ogunmakinwa J, Wasserman AG, Epstein SE (2004) Association of serum antibodies to heat-shock protein 65 with coronary calcification levels: suggestion of pathogen-triggered autoimmunity in early atherosclerosis. Circulation 109:36–41
26. Liu S, Wang X, Li Y, Xu L, Yu X, Ge L, Li J, Zhu Y, He S (2014) Necroptosis mediates TNF-induced toxicity of hippocampal neurons. Biomed Res Int. doi:10.1155/2014/290182
27. Xie J, Shaikh ZA (2006) Cadmium-induced apoptosis in rat kidney epithelial cells involves decrease in nuclear factor-kappa B activity. Toxicol Sci 91:299–308
28. Bonizzi G, Karin M (2004) The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol 25:280–288
29. Kim JH, Kim SJ (2014) Overexpression of microRNA-25 by withaferin a induces cyclooxygenase-2 expression in rabbit articular chondrocytes. J Pharmacol Sci 125:83–90
30. Park MH, Song HS, Kim KH, Son DJ, Lee SH, Yoon DY, Kim Y, Park IY, Song S, Hwang BY, Jung JK, Hong JT (2005) Cobrotoxin inhibits NF-kappa B activation and target gene expression through reaction with NF-kappa B signal molecules. Biochemistry 44:8326–8336
31. Asea A, Kraeft SK, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK (2000) HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6:435–442
32. Chen L, Zhou J, Gao W, Jiang YZ (2003) Action of NO and TNF-α release of rats with cadmium loading in malfunction of multiple system organs. Sheng Li Xue Bao 55:535–540
33. Du Y, Zhu Y, Teng X, Zhang K, Teng X, Li S (2015) Toxicological effect of manganese on NF-kappaB/iNOS-COX-2 signaling pathway in chicken testes. Biol Trace Elem Res. doi:10.1007/s12011-015-0340-5