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RESEARCH ARTICLE

Superoxide dismutase and antioxidant status of hens' granulosa cells exposed to lead and molybdenum

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Özet

Capcarova M, Kolesarova A, Sirotkin AV. Kurşun ve molibdene maruz kalan tavuk granulusa hücrelerinin superoksid dismutaz ve antioksidan durumları. **Eurasian J Vet Sci, 2012, 28, 4, 209-213**

Amaç: Mevcut araştırmanın amacı in vitro kültüre edilen tavuk ovaryum granulosa hücrelerine kurşun (Pb) ve molibden (Mo) uygulaması sonrasında superoksid dismutaz (SOD) ve total antioksidan durum (TAS)'un belirlenmesidir.

Gereç ve Yöntem: Ovaryum granulosa hücreleri farklı dozlarda kurşun asetat/amonyum molibdat uygulaması ile 18 saat inkübe edildi. Kurşun asetat/amonyum molibdat ise faklı dozlarda uygulandı; grup A (0.09/0.09 mg/mL), grup B (0.13/0.13 mg/mL), grup C (0.17/0.17 mg/mL), grup D (0.33/0.33 mg/mL), grup E (0.5/0.5 mg/mL) ve kontrol.

Sonuçlar: Granulosa hücrelerinin antioksidan durumu Pb ve Mo dozuna bağlı olarak değişti. Her iki metalin yüksek dozu SOD aktivitesinde önemli düşmelere neden oldu. Hücrelerin TAS'u deneme gruplarında düşük belirlendi.

Öneri: İz elementler hayvanların üreme sistemi üzerine doğrudan veya dolaylı olarak oksitatif strese neden olarak olumsuz etkilere neden olabilir.

Abstract

Capcarova M, Kolesarova A, Sirotkin AV. Superoxide dismutase and antioxidant status of hens' granulosa cells exposed to lead and molybdenum. **Eurasian J Vet Sci, 2012, 28, 4, 209-213**

Aim: The aim of this study was to determine the activity of superoxide dismutase (SOD) and total antioxidant status (TAS) of ovarian granulosa cells of hens cultured in vitro after lead (Pb) and molybdenum (Mo) administrations.

Material and Methods: Ovarian granulosa cells were incubated with lead acetate/ammonium molybdate administrations as follows: group A (0.09/0.09 mg/mL), group B (0.13/0.13 mg/mL), group C (0.17/0.17 mg/mL), group D (0.33/0.33 mg/mL), group E (0.5/0.5 mg/mL) and the control group without any additions for 18 h.

Results: Antioxidant status of granulosa cells was dependent on Pb and Mo doses. Higher doses of both metals caused significant reduction of SOD activity. TAS of cells was decreased in experimental groups.

Conclusion: Trace elements can adversely affect animal reproductive system and its functions, through either direct or indirect effects on oxidative stress induction.

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Anahtar kelimeler: Granulosa hücreleri, kurşun, molibden, SOD, total antioksidan durum
Keywords: Granulosa cells, lead, molybdenum, SOD, total antioxidant status

► Introduction

The reproductive health of female could be affected by many endogenous and exogenous factors including heavy metals. Female reproductive functions can be affected negatively by exposure to toxic chemicals (Mlynarcikova et al 2005). In recent years, there has been growing interest in the roles of reactive oxygen species (ROS) in female reproduction. ROS are key signals in the initiation of apoptosis in antral follicles and granulosa cells of antral follicles by diverse stimuli, such as exposure to exogenous toxicants, and that antioxidants protect against these stimuli (Devine et al 2012). Recent advances in avian reproduction have focused on the potential of ROS as one of the prime mediators of infertility (Khan 2011). ROS are involved in the modulation of physiological reproductive functions (Agarwal et al 2005). When ROS are overproduced, oxidative stress may develop in the body (Jones 2008). Superoxide dismutase (SOD) serves as front-line antioxidant defence (Scandalios 2005). SOD reacts with superoxide anion radicals to form oxygen and H2O2 (Ho et al 1998).

Trace elements in the environment such as lead (Pb), cadmium, mercury, molybdenum (Mo) can alter steroid production (Stadnicka 1980, Priya et al 2004, Kolesarova et al 2009). Lead is known reproductive toxicant, which accumulate in granulosa cells of the ovary (Nampoothiri and Gupta 2006) and exert a direct influence on granulosa cells functions (Priya et al 2004). The transition elements molybdenum (Mo) is an essential micronutrient for plants, animals, and most micro-organisms (Sigel and Sigel 2002, Mendel 2009). Unavailability of Mo is lethal for the organism (Alhendawi et al 2005). Dietary high Mo (1-1.5 mg/kg) impaired the progression of renal cells from S phase to G(2)M phase obviously, induced renal cell apoptosis (Xiao et al 2011a), and injured immune system of avian broilers (Xiao et al 2011b).

The objective of this study was to determinate the antioxidant status of ovarian granulosa cells of hens after Pb and Mo administration. The effect of various doses of Pb and Mo on total antioxidant status (TAS) and activity of superoxide dismutase of granulosa cells was examined.

► Materials and Methods

• *Preparation, culture and processing of granulosa cells from ovaries*

White Leghorn hens (total n=12) about 500 days old, with an egg laying rate of more than 75%, were held under standard conditions at the Experimental Station of the Slovak Agricultural University in Nitra. Conditions of their care, manipulations and use did correspond the instruction of EC no. 178/2002 and related EC documents, and they were approved by local ethic commission. Birds were decapitated between 9:00 and 11:00 and the largest (F1-F2) follicles were isolated from the ovary. The stage of folliculogenesis was determined by recording the time of the last oviposit and by the size and position of the next ovarian follicle. Granulosa cells were isolated by centrifugation for 10 min at 200xg followed by washing in sterile DMEM/F12 1:1 medium (BioWhittaker™, Verviers, Belgium) and resuspended in the same medium supplemented with 10% fetal calf serum (BioWhittaker™) and 1% antibiotic/antimycotic solution (Sigma, St. Louis, Mo, USA) at a final concentration of 106 cells/mL of medium. Portions of the cell suspension were dispensed to 24-well culture plates (Nunc™, Roskilde, Denmark, 1 mL/well). The well plates were incubated at 38.5 0C and 5% CO2 in humidified air 2 until a 75% confluent monolayer was formed (4 days, determined by haemocytometer). At this point the medium (1 mL/well) was renewed (Kolesarova et al 2009).

• *Lead and molybdenum treatment*

Ovarian granulosa cells were incubated with the 1% antibiotic-antimycotic solution and with lead acetate/ammonium molybdate administrations as follows: group A (0.09 mg/mL), group B (0.13 mg/mL), group C (0.17 mg/mL), group D (0.33 mg/mL), group E (0.5 mg/mL) and the control group without any additions for 18 h. The culture media from well plates were aspirated and cells from plates were manually smashed and lysate was obtained.

• *SOD and TAS analysis*

The activity of antioxidant enzyme SOD and TAS of ovarian granulosa cells was assayed by spectrophotometer Genesys 10 using antioxidant RANDOX kits (Randox Labs., Crumlin, UK) according to the manufacturer's instructions.

• *Statistical analysis*

Each group was represented by four culture wells of cultured granulosa cells. Assays of substances in incubation medium were performed in duplicate. Significant differences between the control and experimental groups were evaluated by one-way ANOVA test analysis of variance using statistical software Sigma Plot 11.0 (Jandel, Corte Madera, USA). The data are expressed as means ± SEM. Differences were compared for statistical significance at the levels $p<0.05$.

► Results

• *The activity of SOD in hens' ovarian granulosa cells after Pb and Mo exposure*

The activity of SOD in granulosa cells of hens after Pb treatment in vitro is shown in Figure 1. In the control group the highest activity of SOD was found. Pb significantly ($p<0.05$) decreased activity of SOD in all experimental groups when compared with the control group. The activity of enzyme increase in E group (the highest administration of Pb) in comparison to remaining experimental groups (A, B, C, and D), how-

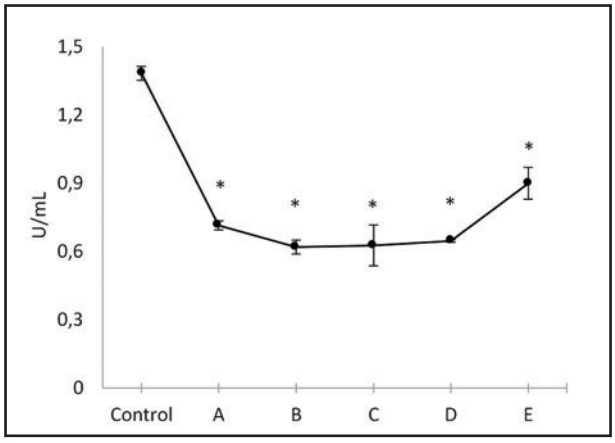

*Figure 1. Effect of lead exposure on SOD activity in ovarian granulosa cells of hens. Control represents lysate without lead administration. Group A received lead acetate at 0.09 mg/mL, group B 0.13 mg/mL, group C 0.17 mg/mL, group D 0.33 mg/mL, and group E 0.5 mg/mL. *Significant differences from Control (p<0.05).*

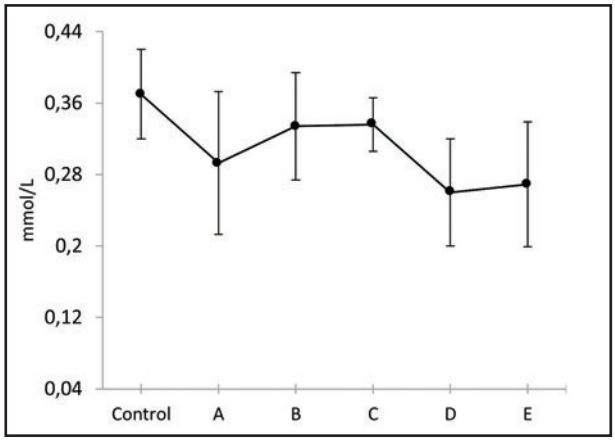

Figure3. Effect of lead exposure on TAS in ovarian granulosa cells of hens. Control represents lysate without lead administration. Group A received lead acetate at 0.09 mg/mL, group B 0.13 mg/mL, group C 0.17 mg/mL, group D 0.33 mg/mL, and group E 0.5 mg/mL. Differences were not significant (p>0.05).

ever without significant differences (p>0.05).

Significant changes (p<0.05) in activity of SOD in ovarian cells of hens after Mo treatment were found (Figure 2). The smallest dose of Mo had no effect on investigated enzyme. Higher doses of Mo (groups B, C, D, and E) significantly (p<0.05) lowered the activity of SOD against the control group. The activity in the groups with the highest doses of Mo (D and E) showed increase in SOD activity when compared with remaining experimental groups but without significant confirmation (p>0.05).

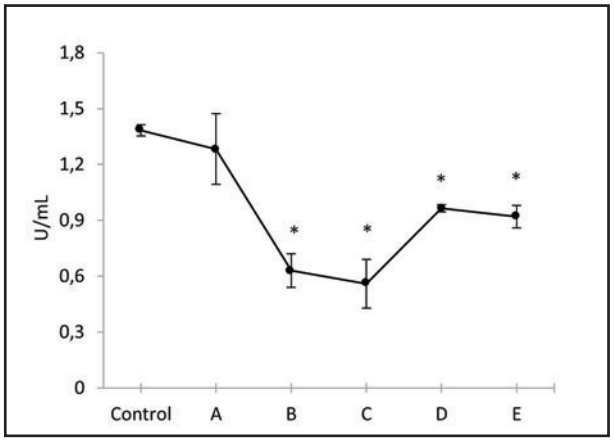

*Figure 2. Effect of molybdenum exposure on SOD activity in ovarian granulosa cells of hens. Control represents lysate without molybdenum administration. Group A received ammonium molybdate at 0.09 mg/mL, group B 0.13 mg/mL, group C 0.17 mg/mL, group D 0.33 mg/mL, and group E 0.5 mg/mL. *Significant differences from Control (p<0.05).*

• *TAS of hens' ovarian granulosa cells after Pb and Mo exposure*

The TAS of granulosa cells of hen are presented in Figures 3, 4. The highest TAS was determined in the control group. In all experimental groups (lead treatment) the values of TAS were decreased in comparison with the control group. The lowest values were found in the groups with highest doses of Pb (D and E). Statistical analyses showed that the differences among the groups remained insignificant (p>0.05).

► Discussion

The presence of harmful substances such as heavy metals in poultry feed is sometimes unavoidable (Kan 1994). The exposure of hens or chickens to various metals caused alteration of zootechnical parameters (Arpasova et al 2007) as well as imbalance in internal milieu (Capcarova et al 2008, Kolesarova et al 2008)

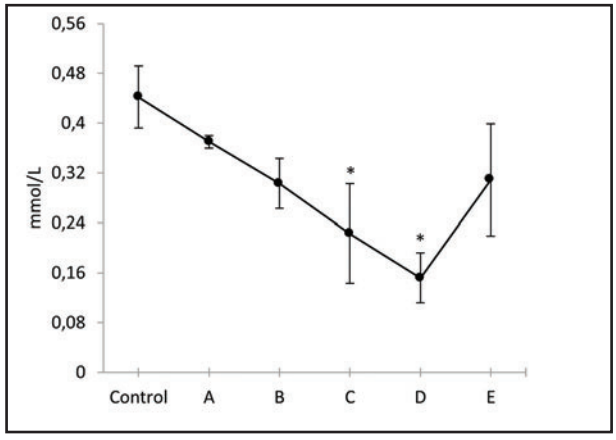

*Figure4. Effect of molybdenum exposure on TAS in ovarian granulosa cells of hens. Control represents lysate without molybdenum administration. Group A received ammonium molybdate at 0.09 mg/mL, group B 0.13 mg/mL, group C 0.17 mg/mL, group D 0.33 mg/mL, and group E 0.5 mg/mL. *Significant differences from Control (p<0.05).*

and hormonal release by ovarian granulosa cells of hens (Kolesarova et al 2009). Lead was capable of accumulating in porcine ovarian granulosa cells of rats (Nampoothiri and Gupta 2006) and sheep (Bires et al 1995). In our study, lead and molybdenum treatment significantly decreased the activity of SOD in porcine granulose cells. From our earlier investigation

emerged that isolated ovarian cells of hens were able to survive in culture after experimental Pb and Mo administration. Secretory activities of granulosa cells were dependent on Pb and Mo doses (Kolesarova et al 2009). Lead may have negative effect on porcine ovarian granulosa cells when added to culture (Capcarova et al 2009).

In our previous results porcine granulosa cells were capable of maintaining stable SOD activity despite various doses of Pb (Capcarova et al 2009). Molybdenum is an essential trace element and it plays an important role in cell function. Our previous study revealed dose-dependent regulation of IGF-I of porcine granulosa cells after experimental Mo administration in vitro (Kolesarova et al 2011). Du Plessis et al (1999) reported occurring of behavioural anoestrus in sheep fed a high molybdenum diet, in size reduced ovaries, decreased response to superovulation. In another study (Panneerselvam and Govindasamy 2004) the oral administration of molybdate to diabetic rats significantly reduced lipid peroxidation and increased the activity of SOD.

Total antioxidant status is defines as the sum total of endogenous and food derived antioxidants of the extracellular fluids of an individual (Miller et al 1993). TAS seems to correlate with decreased fertilization potential (Tatone et al 2008). Our previous study also revealed that TAS of porcine granulosa cells was decreased by virtue of lead (Capcarova et al 2009). TAS decreased in proportion to dose of Mo (except E group). Generally, Mo exposure caused decrease in TAS of granulosa cells, significantly ($p<0.05$) in C and D group when compared to the control group. The lowest value was measured in D group. Eidi et al (2011) found that sodium molybdate can protect the liver against induced oxidative damage in rats.

► Conclusions

Lead and molybdenum exposure may have negative effect on ovarian granulosa cells of hens. The activity of SOD significantly decreased in experimental groups in comparison to the control group. TAS was decreased after both metals treatment, significantly in case of molybdenum in doses 0.17 mg/mL and 0.33 mg/mL. The research on the field of antioxidant system and protection of various cells against environmental pollution will be worthy of further investigation.

► Acknowledgments

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