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REGULAR ARTICLE

THE EFFECT OF CADMIUM INJECTED *IN OVO* ON MINERALS LEVEL IN TISSUES OF ONE-DAY OLD CHICKS

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ABSTRACT

The distribution of cadmium injected *in ovo* on day 4. of incubation in tissues of one-day old chicks was examined for the first time by the LA-ICP-MS method . The effect of cadmium on metabolism of essential elements in tissues was also investigated. The kidneys and liver were the main target organ for cadmium ($P<0.05$) in chicks, but far more intensive accumulation in reproductive tissues was observed. The fluctuations in the levels of majority of studied elements were found but significant differences ($P<0.05$) were observed only for manganese, calcium , and selenium. The most intensive increase of selenium were observed in gonads, probably as the defense factor against oxidative stress induced by cadmium.

Keywords: cadmium, *in ovo*, chicks, tissues, LA-ICP-MS, selenium

INTRODUCTION

Cadmium (Cd) is currently one of the most important toxic metals in occupational and environmental toxicology, due to an increasing industrial use in electroplating, as a pigment and a plastics stabilizer, and in nikiel-Cd batteries (**Culliane *et al.*, 2009**). The contamination of soil and plant may lead to enhanced dietary Cd. This heavy metal is absorbed in significant quantities from cigarette smoke, and is known to have numerous undesirable effects in both experimental animals and humans, targeting the kidneys, liver and vascular system in particular (**Thompson and Banningan, 2008**). Moreover, the biochemical alterations occur prior to morphological changes in the organs, including the changes in certain enzyme levels in extracellular fluids and mineral homeostasis of organism (**Martelli *et al.*, 2006; Nordberg, 2010**).

The adverse effect on fertility as well as early embryo development in a wide variety of animals and human-smokers have been intensively studied. Numerous studies have also investigated the teratogenic effects of Cd in chick embryos using both *in ovo* and shell-less culture experiments (**Thompson and Banningan, 2008**; **Culliane *et al.*, 2009; Rahaman *et al.*, 2009; Dżugan et al., 2011).** Although, the presence of carry-over effect of the maternal Cd into avian eggs is still under discussion, the embryo model is commonly used for testing the Cd embryotoxicity (**Rahman *et al.*, 2009)**.

A number of mechanisms of Cd toxicity have been suggested, including ionic and molecular mimicry, interference with cell adhesion and signaling, oxidative stress, apoptosis and cell cycle disturbances (**Thompson and Banningan, 2008; Czeczot and Skrzycki, 2010**). Moreover, protective effect of some divalent cations, such as zinc, calcium, magnesium, manganese, against cadmium toxicity have been frequently demonstrated (**Bridges and Zalups, 2005**; **Culliane *et al.*, 2009)**. Similarly, selenium is an non-metallic essential element for human and animals and is believed as the antagonist of cadmium, probably due its protective role in oxidative stress.

Despite the fact that numerous study demonstrated a protective effect of antagonists co-administered with cadmium, data on the cadmium interference in metabolism of essential metals are very limited. Recently, LA-ICP-MS (Laser Ablation Inductively Coupled Plasma Mass Spectrometry) as the most sensitive technique for imaging of metals, metalloids, and non-metals on biological tissues has been proposed (**Becker *et al.*, 2008**). The method is very advantageous due to the relatively low instrumental costs and high sample throughput, high sensitivity, accuracy, and precision of the analytical data. Detection limits of imaging LA-

ICP-MS were observed at the range $\mu g.g^{-1}$ down to the low $ng.g^{-1}$. The method is very advantageous due to the relatively low instrumental costs and high sample throughput, high sensitivity, accuracy, and precision of the analytical data. LA-ICP-MS can be employed as a sensitive inorganic MS technique for the imaging of essential and toxic metals (such as Cu, Zn, Pb, Th, and U- often at trace concentration level) and also for the mapping of metalloids (Se) and non-metals (P, S, C, I) in microtome thin tissue sections (optimum at 20 μm thickness) of tissues (**Richardson *et al.*, 2001; Becker *et al.*, 2008**).

In this study for the first time the distribution of Cd injected *in ovo* in tissues of one-day old chicks and Cd effect on the levels of essential elements were studied using LA-ICP-MS method.

MATERIAL AND METHODS

Chicks

Hatching broilers' eggs (n=100) with live embryos were injected with cadmium on the 4th day of incubation. The dose of 6 μg Cd (as 50 μl of $CdCl_2$ in saline solution) was introduced into egg albumen (n=50, Cd-group), whereas the control eggs (n=50) were injected with saline. The incubation was continued in standard conditions (E1-E18: T = 37.8°C, RH = 50%; E19-E21: T = 37.2 ± 0.1°C, RH = 70%). Eggs were candled on day 8. and 18. of incubation and those with dead embryos were eliminated. Immediately after hatching, chicks were weighed. Tissues (liver, kidney and gonads) were removed (n=5 per group) directly after decapitation. All samples were stored at -80°C until analyses.

Metal examination

A Laser Ablation Inductively Coupled Plasma Mass Spectrometer (LA-ICP-MS) was used for determination of cadmium in the tissues samples. All samples before measurements were dried in room conditions on glass slides and put into the laser ablation chamber –Super cell (New Wave UP 213 nm). When the laser started ablating the sample, the particles of the sample were swept in a carrier gas (He) and moved towards the plasma of the ICP-MS system (Thermo Fisher Scientific X series2 ICP MS). The ablated material were ionized and analyzed similarly to any liquid sample by ICP MS. For cadmium determination in tissue, the measuring parameters were optimized in the following manner: laser energy 50%, repetition

rate 20Hz, dwell time 5s and spot size 30 µm. The instrumental response was measured in counts per second (Cps). A measurement was done five times for each sample on different areas of the sample surface.

Statistical analysis

Differences between experimental and control group were assessed using Kruskal-Wallis and Mann-Whitney U-tests ($P<0.05$). Coefficients of correlation were calculated using Pearson's correlation analysis. All statistical analyses were completed using Statistica 8. (Statsoft 2007).

RESULTS AND DISCUSSION

Hatching results

Hatchability of the chicks after cadmium *in ovo* injection in E4 significantly decreased from 62% in the control group to 40% in the group exposed to 6 µg Cd/egg (Table 1). These results are better than our previous observations (**Dżugan *et al.*, 2011**), where we obtained (with the same Cd dose) only 45 and 19.5% chicks in control and Cd-injected groups, respectively.

Table 1 Hatching results after *in ovo* injection of Cd (II) ions on day 4 of incubation (E4)

Group		Control	Experimental
Injected Cd dose [µg/egg]		0	6
Group size (egg number)	n	50	50
Deaths after injection (E4-E7)	%	16	28*
Deaths from E7 to E20	%	22	32*
Hatching	%	62	40*
Chick weight [g]	mean±SD	42.6±2.1	43.8±4.4

*statistical differences ($P<0.05$)

In the present study, we observed lower mortality caused by mechanical interference to the egg content, about 16% of embryos to die within 48 h of *in ovo* injection in control group in comparison to 30% mortality noted in previous experiment (**Dzugan *et al.*, 2011**). It can be assumed that the observed increase in embryo mortality (about 22% vs. control group)

is caused by the embryotoxic effect of cadmium (**Thompson and Bannigan, 2008).** During the experiment, *in ovo* administration of cadmium ions had no effect (P>0.05) on the body weight of hatched chicks.

Cadmium distribution

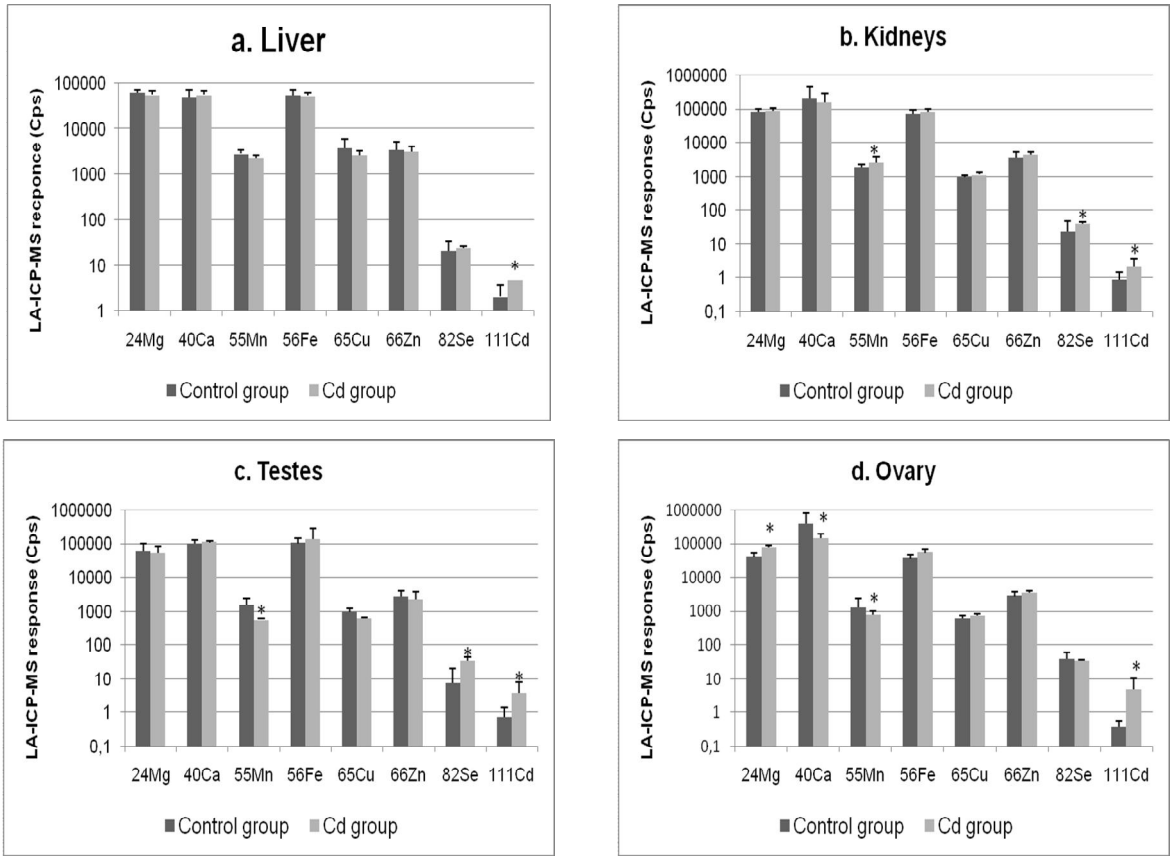

Figure 1 LA-ICP-MS examination of metal content in tissues of one-day old chicks exposed to cadmium during embryogenesis: (a) liver, (b) kidneys, (c) testes and (d) ovary.
*Significant differences between Control and Cd groups (P<0.05)

It has been confirmed that cadmium administered *in ovo* was accumulated (P<0.05) in main target organs, i.e. kidneys (fig. 1a) and liver (fig 1b). Moreover, high accumulation in gonads was observed (figs 1c and 1d), but in both tissues great variability coefficient (>100% for Cd group) was noted. However, kidney and liver are recognized as a main Cd storage tissues and are mostly studied, the observation of cadmium accumulation in gonads is rarely conducted. In guinea pigs, during chronic exposure (by i.p. administration) cadmium was accumulated mainly in kidney (46-61%), liver (15-30%), and gonads (15-17%). Moreover

higher dynamic of cadmium accumulation with increasing dose in ovaries than in the testes was documented (**Munga *et al.*, 2010**). Thus, our results are in agreement with these findings.

Metal-metal interactions

Experimental evidences indicate that Cd may interact with membrane transporters involved in the uptake of Ca, Fe, Zn and Mn through the process of "ionic mimicry" (**Chmielnicka and Sowa, 1996; Bridges and Zulups, 2005; Marteli *et al.*, 2006).** Thus, the effect of Cd administered *in ovo* on metabolism of these elements was expected. Although, fluctuations in the level of all elements caused by cadmium injected *in ovo* were observed, when compared with control group (Fig. 1a÷d), but significant changes ($P<0.05$) were found in several cases only. Cd-induced increase of manganese level in kidneys was demonstrated and simultaneous its decrease in the gonads. The adverse effect of cadmium on calcium level was observed in ovary ($P<0.05$) and in kidneys. Surprisingly, cadmium was no affected the zinc level.

The most significant effect induced by cadmium was observed for selenium. The concentration of this element was enhanced in all examined tissues, and only in the case of liver the change was non-significant. A similar dependence was observed previously in kidney and liver of rats exposed to Cd (**Ognjanović *et al.*, 2008; Toman *et al.*, 2009**). The authors suggested that increase in cadmium uptake mobilizes the selenium storage in the tissues which can be target organ for cadmium. The mechanism of protection may be connected with induction of synthesis of glutathione peroxidase (GPx). This selenium-dependent enzyme is one of the main protective agents in cell defence against oxidative stress. Thus, replacement selenium load from whole body to gonads may protect sensitive reproductive tissues against cadmium toxicity.

Due to small changes in essential metals level observed, Cd-metal interactions were mostly non-significant (Table 2). The negative correlations between Cd-Mn in liver and testes, whereas positive relation for Se-Cd in kidneys and testes, and for Cd-Cu in ovary were demonstrated.

Table 2 Pearson's correlation for metal-metal concentration in tissues of examined one-day old chicks

Tissue	Person's coeficient (r) for metals concentrations in tissues of chicks						
	Cd-Mg	Cd-Ca	Cd-Mn	Cd-Fe	Cu-Cd	Cd-Zn	Cd-Se
Liver	-0.324	0.265	-0.598*	-0.345	-0.350	0.164	0.381
Kidneys	0.450	0.171	0.329	0.498*	0.082	0.354	0.429*
Testes	0.161	0.061	-0.433*	-0.275	-0.368	0.282	0.811*
Ovary	0.343	-0.353	-0.096	0.271	0.606*	-0.070	0.228

CONCLUSION

This is first study of using LA-ICP-MS method to assess the distribution of heavy metals in tissues of chicks exposed *in ovo* to cadmium. It was demonstrated that cadmium administered *in ovo* was accumulated mainly in chicks kidneys and liver, but intensive cadmium uptake in reproductive tissues of both male and female birds were observed.

Moreover it was shown, that LA-ICP-MS is an effective tool for mapping the distribution of administered cadmium dose in tissues of experimentally exposed animals. It provides an attractive alternative for the traditional "wet" methods, especially when numerous samples are analyzed but to fully use it requires quantitative calibration.

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