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The Affect of Cobalt Salts on Some Weight Indices in Developing Mice

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Although cobalt is an essential trace element long-term exposure and large amounts of its salts can have deleterious effects on humans and animals. Pregnant balb/c mice in late gestation were subjected to cobalt chloride ($CoCl_2.6H_2O$) or cobalt EDTA (Co-EDTA) treatment at daily doses of 75 mg/kg or 125 mg/kg. Cobalt compounds were dissolved and obtained from drinking tap water. Sodium EDTA (Na-EDTA) and pure tap water were used as controls. The newborn pups were sacrificed on days 18, 25 and 30 which correspond to different stages of development. Mice were weighed weekly and the experimental cobalt concentration was adjusted accordingly. Preliminary results showed that mice treated with cobalt salts ($CoCl_2$ and Co-EDTA) have smaller body weight compared to the control group. Liver weight was increased in the Co-EDTA-treated mice for both doses in all experimental groups. Spleen and liver weight was increased in case of high dose $CoCl_2$-treated mice. Spleen weight was the largest in high dose $CoCl_2$ –treated mice compared to all other groups. Liver weight of mice treated with Co-EDTA was the largest in all experimental groups compared to that induced by the other substances and in the control. The experimental results show that organic and inorganic cobalt salts affect body and organ weight.

Key words: mice, cobalt salts, liver, spleen, indices.

Introduction

Heavy metals are widely spread in the environment, food and water and exposure to them is unavoidable. Elevated values of the heavy metals' concentration in various organs of humans, animals and aquatic fish are measured [12].

Although cobalt is as essential trace element long-term exposure and large amounts of its salts can have deleterious effects on humans and animals. Cobalt (II) accumulates in organs such as spleen, kidney, heart and liver [1]. Its salts are shown to affect body weight of patients and experimental animals but the mechanism remains to be elucidated. Data show significant weight loss, as well as decreased food and water consumption in diabetic rats treated with cobalt chloride [10, 11]. No data were found for

the influence of cobalt compounds on animals in different stages of development after long-term treatment.

Ethylenediamine tetraacetic acid (EDTA) is used in medicine, molecular biology and biochemistry; as anticoagulant for blood samples and decalcifying agent in histopathology, in non-alcoholic beverages, etc. Experiments with animals though show that EDTA exhibits cytotoxic and weakly genotoxic effects. Oral exposure causes reproductive and developmental effects as well.

The *aim* of the present study is to investigate the effect of cobalt compounds ($CoCl_2$ and Co-EDTA) on some somatic indices in developing mice – body weight, liver and spleen weight.

Materials and Methods

Complex synthesis

All chemicals and solvents used were of AR grade. Co-EDTA was synthesized according to modified literature procedures [3, 8], namely adding slowly solution of $Co(NO_3)_2.6H_2O$ (0.291 μg, 1 mmol in 5 ml H_2O) to a mixture containing Na_2EDTA (186 mg, 0.5 mmol in water) and tetraethyl ammonium hydroxide (Et_4NOH) (180 ml, 0.5 mmol, 40% in water) resulting in formation of Co-EDTA. The latter was precipitated as pink suspension with acetone. Then the vessel was covered with parafilm and after approximately a week formation of pink crystals was observed. They were studied using X-ray diffraction method proving a composition of $[Co(H_2O)_4$ (Co-EDTA)$]_n$ with known structure – orthorhombic space group $Pna2_1$ (CCDC Ref:COEDTA) [8].

The structural data was collected on an Oxford Diffraction Sapphire 2 CCD diffractometer with graphite – monochromated Cu-Kα radiation.

Animal model

Pregnant balb/c mice in late gestation were subjected to cobalt chloride ($CoCl_2.6H_2O$) or cobalt EDTA (Co-EDTA) treatment at daily doses of 75 mg/kg or 125 mg/kg which continued until day 30 of the newborn mice. Cobalt compounds were dissolved and obtained from drinking tap water. Sodium EDTA (Na_2EDTA) and pure tap water were used as controls. Animals were fed a standard diet and had access to food *ad libitum*. Mice were maintained in the institute's animal house at 23°C ± 2°C and 12:12 h light-dark cycle in individual standard hard bottom polypropylene cages to ensure that all experimental animals obtained the required dose of cobalt compounds.

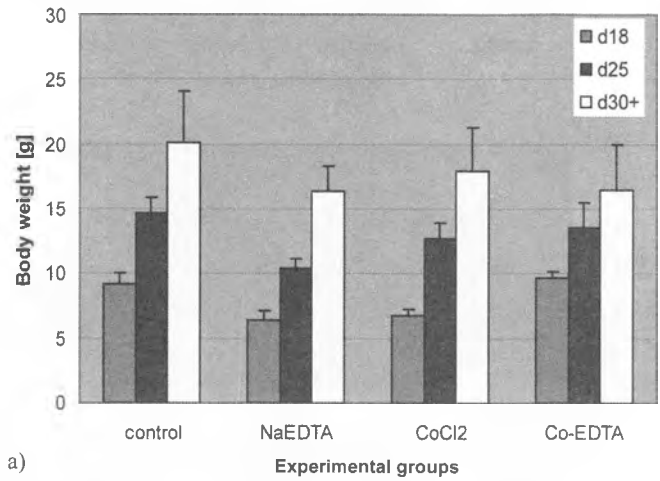
The newborn pups (5 per group) were sacrificed on days 18, 25 and 30 which correspond to different stages of development. Mice were weighed weekly and the experimental cobalt concentration was adjusted accordingly. After the animals were sacrificed liver and spleen were removed and weighed. Liver and spleen indices – liver/body weight (L/BW) and spleen/body weight (S/BW) were calculated. Significance was determined using Student's *t*-test at $p<0.05$.

The studies were approved by the Ethics Committee of the Institute of Experimental Morphology, Pathology and Anthropology with Museum – Bulgarian Academy of Sciences.

Results and Discussion

The experimental results showed that cobalt salts (Co-EDTA and $CoCl_2$) affect body and organ weight. Mice treated with $CoCl_2$ and Co-EDTA have smaller body weight compared to the control group (Fig. 1a, b). Data are in agreement with Garoui et al. [2] showing retarded weight gain in suckling rats. These results suggest that perinatal and postnatal exposure of rats and mice to cobalt, retards the growth of their pups, possibly due to transfer of Co^{2+} through placenta and milk.

Treatment with $CoCl_2$ and Co-EDTA affects spleen and liver weight as well. The effect depends on the type of compound used, dose and time duration. Results showed that day 18 mice are the most sensitive to cobalt treatment. Our results are not in agreement with those of Mazur [7] who shows that $CoCl_2$-treated rats do not have increased spleen weight. In our experimental model, exposure to high dose $CoCl_2$ (125 mg/kg)



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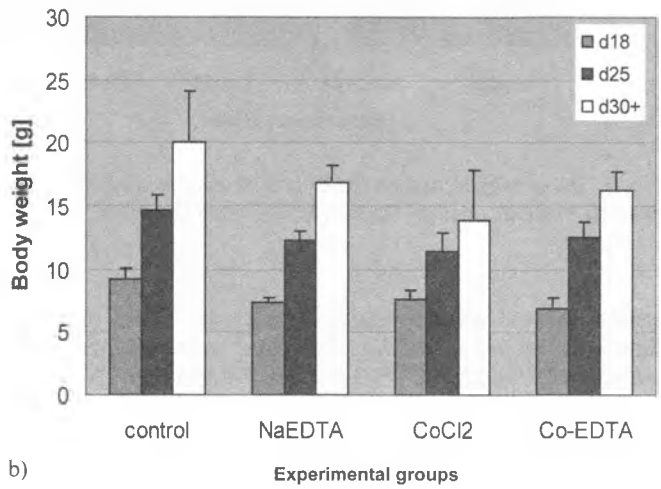

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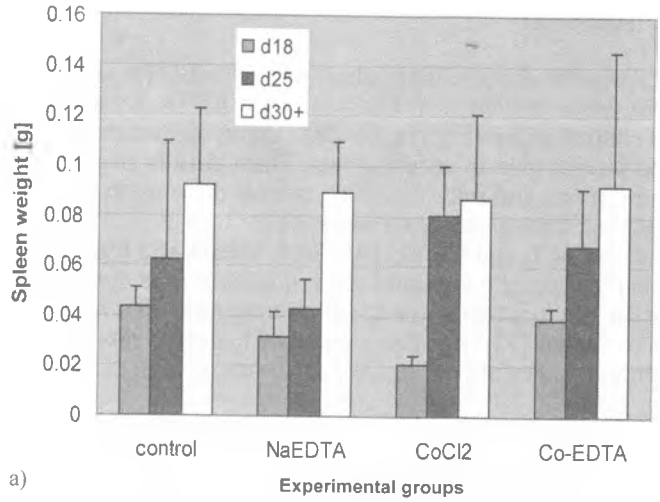
Fig. 1. Body weight of day 18, 25 and 30 mice treated with low dose (a), and high dose (b) Na_2EDTA, $CoCl_2$ and CoEDTA



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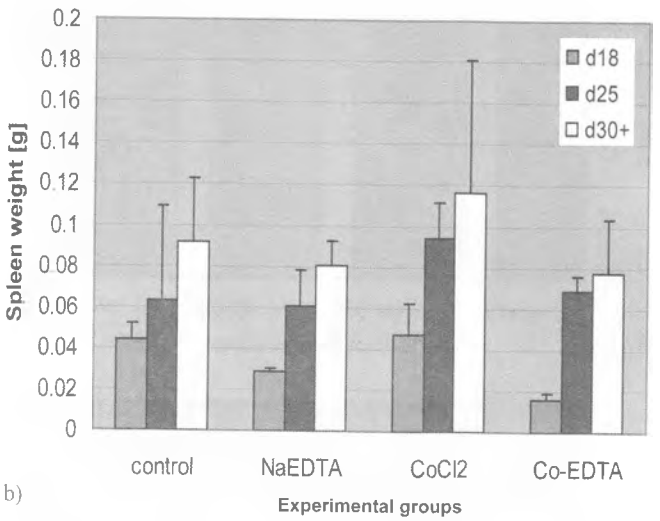
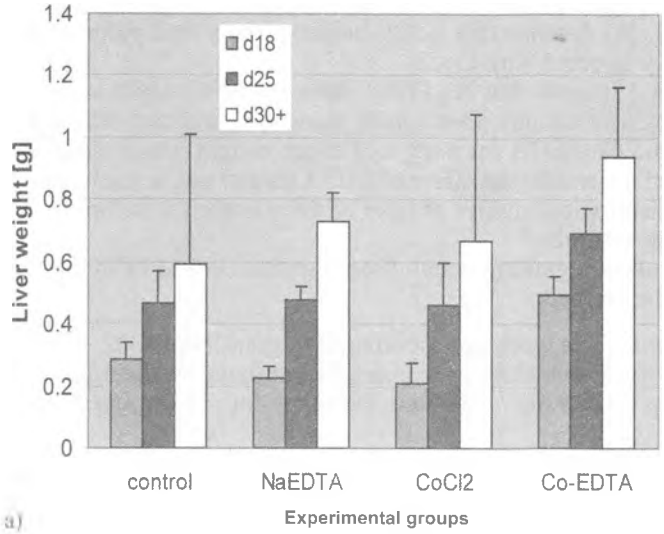


b)

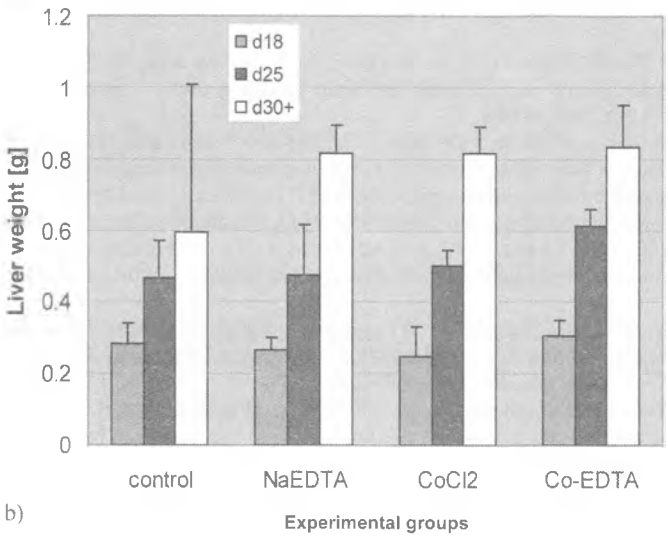
Fig. 2. Spleen weight of day 18, 25 and 30 mice treated with low dose (a), and high dose (b) Na_2EDTA, $CoCl_2$ and CoEDTA

caused an increase in mouse spleen weight [Fig. 2a, b]. When mice were treated with Co-EDTA though, spleens were smaller compared to the control samples. When the effects of high dose $CoCl_2$ and Co-EDTA for day 18 and day 25 mice were compared, the differences were significant ($p<0.004$ and $p<0.03$, respectively). Spleen index (calculated as a ratio to body weight) was significantly decreased ($p<0.004$) in low dose $CoCl_2$-treated day 18 mice and significantly increased ($p<0.003$) in mice treated with the high dose. High dose Co-EDTA also caused a significant decrease ($p<0.001$) in spleen index in day 18 mice.

Treatment with high dose $CoCl_2$ increased liver weight in day 25 and day 30 mice (Fig. 3a, b). Similar results were obtained for mice subjected to both doses (low and



a)



b)

Fig. 3. Liver weight of day 18, 25 and 30 mice treated with low dose (a), and high dose (b) Na_2EDTA, $CoCl_2$ and CoEDTA

high dose) Co-EDTA for days 18, 25 and 30. Low dose Co-EDTA increased significantly liver weight in day 18 ($p<0.001$) and day 25 ($p<0.001$) mice compared to that of $CoCl_2$-treated mice. For the high dose significance was found only for day 25 mice ($p<0.01$). Liver index (calculated as a ratio to body weight) was significantly increased in day 18 and day 25 when treated with either low or high dose Co-EDTA. The results suggest stronger effect of Co-EDTA on liver compared to $CoCl_2$ which is known to induce oxidative stress in rat liver leading to a significant increase in heme oxygenase-1 activity [4]. Garoui et al. [2] show that $CoCl_2$ treatment leads to infiltration of mononuclear cells, indicating the presence of inflammatory reactions and vascular congestion in livers of rat pups and dams. It also exhibits *in vitro* a protective effect on apoptotic

cell death in hepatic cell line HepG2 reducing DNA fragmentation [9]. On the other hand, Liu et al. [6] demonstrate $CoCl_2$-hepatotoxicity and pulmonary edema in mice intraperitoneally injected with $CoCl_2$.

Hiers et al. [5] show that Na_2EDTA does not affect organ weight as a percentage of body weight in ruminants. Our results showed significant differences in the effects of Na-EDTA and Co-EDTA for body and organ weight which suggests that for proper interpretation of the results the affect of EDTA should not be excluded. Our preliminary results of the histological studies of liver of mice treated with Na_2EDTA show changes compared to the controls.

Further studies regarding cobalt bioaccumulation and its cytotoxicity in liver and spleen will be performed.

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References

1. Ayala-Fierro, F., J. M. Firriolo, D. E. Carter. Disposition, toxicity, and intestinal absorption of cobaltous chloride in male Fischer 344 rats. – J. Toxicol. Environ. Health A., **56**, 1999, 571-591.
2. Garoui, E. M., H. Fetoui, F. A. Makni, T. Boudawara, N. Zeghal. Cobalt chloride induces hepatotoxicity in adult rats and their suckling pups. – Exp. Toxicol. Pathol., 2009, doi:10.1016/j.etp.2009.09.003.
3. Gomez-Romero, P., G. B. Jameson, N. Casan-Pastor, E. Coronado, D. Beltran. Low-dimensional bimetallic ordered systems: synthesis and characterization of the isomorphous series of the cobalt nickel complexes CoxNi2-xEDTA.2H_2O. Crystal structure of Co2EDTA.2H_2O and preferential site occupation in CoNiEDTA.H_2O. – Inorganic Chemistry, **25**, 1986, 3171-3176.
4. Gonzales, S., A. H. Polizio, M. A. Erario, M. L. Tomaro. Glutamine is highly effective in preventing in vivo cobalt-induced oxidative stress in rat liver. – World J. Gastroenterol., **11**, 2005, 3533-3538.
5. Hiers, J. M., W. J. Miller, D. M. Blackmon. Effect of Dietary Cadmium and Ethylenediaminetetraacetate on Dry Matter Digestibility and Organ Weights in Zinc Deficient and Normal Ruminants. – J. Dairy Sci., **51**, 1968, 205-209.
6. Liu, W., M. Guo, Y. B. Xu, D. Li, Z. N. Zhou, Y. L. Wu, Z. Chen, S. C. Kogan, G. Q. Chen. Induction of tumor arrest and differentiation with prolonged survival by intermittent hypoxia in a mouse model of acute myeloid leukemia. – Blood, **107**, 2006, 698-707.
7. Mazur, A. Metabolism of the Stimulated Rat Spleen. I. Ferrochelatase activity as an index of tissue erythropoiesis. – J. Clin. Invest., **47**, 1968, 2230-2238.
8. McCandlish, E. F. K., T. K. Michael, J. A. Neal, E. C. Lingafelter, N. J. Rose. Comparison of the structures and aqueous solutions of [(o-phenylenediaminetetraacetato(4-)]cobalt(II) and [ethylenediaminetetraacetato(4-)]cobalt(II) ions. – Inorganic Chemistry, **17**, 1978, 1383-1394.
9. Piret, J. P., C. Lecocq, S. Toffoli, N. Ninane, M. Raes, C. Michiels. Hypoxia and CoCl2 protect HepG2 cells against serum deprivation- and t-BHP-induced apoptosis: a possible anti-apoptotic role for HIF-1. – Exp. Cell Res., **295**, 2004, 340-349.
10. Vasudevan, H., J. H. McNeill. Chronic cobalt treatment decreases hyperglycemia in streptozotocin-diabetic rats. – BioMetals, **20**, 2007, 129-134.
11. Ybarra, J., A. Behrooz , A. Gabriel , M. H. Koseoglu , F. Ismail-Beigi. Glycemia-lowering effect of cobalt chloride in the diabetic rat: increased GLUT1 mRNA expression. – Mol. Cell Endocrinol., **133**, 1997, 151-160.
12. Арнаудова, Д., Е. Томова, И. Велчева, А. Арнаудов. Проучване съдържанието на олово, цинк и кадмий в някои органи на риби от сем. Cyprinidae и сем. Percidae в язовирите „Студен кладенец“ и „Кърджали“. – Юбилейна научна конференция по Екология (Сборник с доклади), Ред. И. Велчева, А. Цеков, Пловдив, 2008, стр. 327–335.