EFFECT OF CHRONIC EXPOSURE TO COBALT(II) COMPOUNDS ON ORGANS' WEIGHT INDICES

Yordanka Gluhcheva, Maria Madzharova, Juliana Ivanova

Abstract. Cobalt's (Co) wide use in the industry, in medical devices, as food perservative, in consmetics requires detailed study on its biological effects. The aim of the study was to elucidate the effect of chronic treatment with cobalt(II) compounds – cobalt chloride ($CoCl_2$) and cobalt-EDTA (Co-EDTA) on organ weight indices in immature and mature mice. Pregnant ICR mice were treated daily with 75 mg/kg b.w. or 125 mg/kg b.w. of $CoCl_2$ or Co-EDTA until day 90 of the newborn mice. The compounds were dissolved in regular tap water. The control mice obtained regular tap water. All experimental animals obtained food *ad libitum*. On day 25 pn the newborn mice were placed in individual cages and the treatment continued until day 90. Each week mice were weighed to adjust the dose. At different periods – day 18, 25, 30, 45, 60 and 90 mice were sacrificied. Spleens, liver and kidneys were excised, weighed and organ weight indices - spleen index (SI), liver index (LI) and kidney index (KI) calculated. Chronic exposure to Co(II) compounds resulted in alterations in organ indices – SI, LI, KI. $CoCl_2$ increased SI in immature mice, while Co-EDTA affected the spleen mainly of mature mice. Co exposure led to an increase in LI in Co-EDTA-treated mice. The index decreased when $CoCl_2$ was administered. Kidney index (KI) on the other hand was significantly increased in day 30 mice after treatment with $CoCl_2$. The compound had little or no effect on KI of mature animals. The effect of Co-EDTA was diverse and no clear tendency was observed. The results indicate that the biological effects of Co(II) depend on the type of compound, the duration of exposure and as well as on the age of the experimental animals.

Key words: mice, in vivo model, cobalt chloride, cobalt-EDTA, organ weight indices

INTRODUCTION

The exposure to cobalt (Co) from industry and surgical implants requires thorough studies for the biological effects of the metal ions. For the general population diet (meat, vegetables, drinking water) is the main source of Co. The average daily intake of cobalt ranges from 5-45 μg with relatively high concentrations of the metal occurring in fish and in vegetables [2]. Treatment with Co enhances erythropoietin production, hypoxia-inducible factor (HIF) synthesis, improves tubulointerstitial injuries in the kidneys [9].

Studies on long-term exposure of laboratory animals to the metal ions show that they accumulate in organs such as kidney, liver, spleen, heart, stomach, intestines, muscle, brain and testes [1]. This suggests possible alterations in organ weight indices. There ape lack of data regarding changes in organ weight indices after acute or chronic metal exposure.

The aim of the study is to elucidate the effect of chronic treatment with cobalt(II) compounds – cobalt chloride ($CoCl_2$) and cobalt-EDTA (Co-EDTA) on organ weight indices in immature and mature mice.

MATERIALS AND METHODS

Pregnant ICR mice were treated daily with 75 mg/kg b.w. or 125 mg/kg b.w. of $CoCl_2$ or Co-EDTA until day 90 of the newborn mice. These doses are 50% and 83% of the dose, shown to stimulate erythropoiesis [7]. The compounds were dissolved and obtained from drinking tap water. The mothers were placed in individual cages to ensure that each obtained the required dose. Our previous experience has shown that each mouse drinks approximately 8 ml water/day, therefore the required dose was dissolved in 8 ml per mouse per day. Animals were fed a standard diet and had access to food *ad libitum*. Mice were maintained in the institute's animal house at 23ºC ± 2ºC and 12:12 h light-dark cycle in individual standard hard bottom polypropylene cages. On day 25 pn the newborn mice were placed in individual cages and the treatment continued until day 90. Mice were weighed weekly to adjust the experimental cobalt concentration. At different periods – day 18, 25, 30, 45, 60 and 90 mice were sacrificed. Spleens, liver and kidneys were excised, weighed and organ weight indices - spleen index (SI), liver index (LI) and kidney index (KI) calculated. The indices were calculated as a ratio of organ weight to body weight. All changes were compared to control samples of age-matched mice drinking the same quantity tap water.

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

STATISTICAL ANALYSIS

The obtained results are presented as mean values ± Standard Deviation (SD). Statistical between the experimental groups is determined using Student's *t*-test. Difference is considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Chronic treatment with Co(II) compounds affected body weight of exposed animals suggesting possible alterations in organ weight indices as well. According to Cesta the ratio of splenic weight to body weight remains fairly constant regardless of age [3].

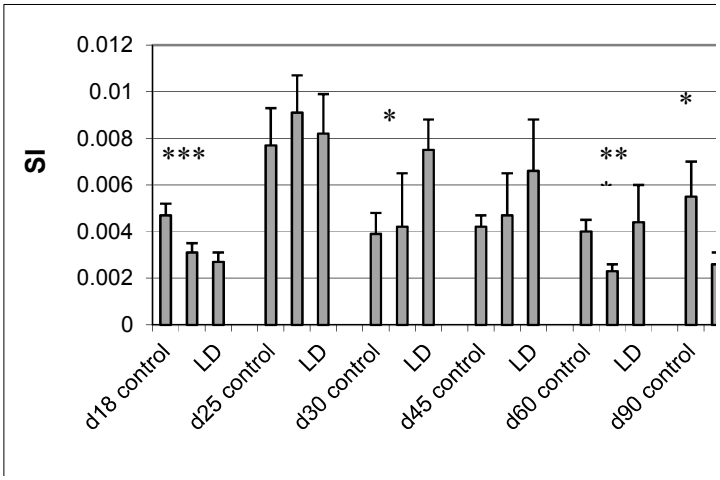

Fig. 1. Spleen index (SI) of mice treated with low dose (75 mg/kg b.w.) $CoCl_2$ and Co-EDTA. "LD" represents low dose Co-EDTA. Each column represents mean ±SD, *n*=5. Asterisk (*) represents statistical difference ($p<0.01$) and triple asterisk (***) represents statistical difference ($p<0.001$).

Treatment with low dose $CoCl_2$ increased SI ~ 1-fold in day 25, 30 and 45 mice. Comparison between the effects of both low doses (Fig. 1) showed that chronic exposure to 75 mg/kg b.w. Co-EDTA induced an increase from ~ 1.1 (day 90) to 1.9 (day 30)-fold increase in SI of treated mice. Significant differences between both compounds were found for days 30, 60 and 90.

Exposure to the high dose of both compounds showed that $CoCl_2$ decreased SI in immature mice (Fig. 2). Treatment with 125 mg/kg b.w. Co-EDTA also decreased SI in day 18 and day 25 mice but increased the index (~1.3-fold) in day 30 animals. The highest increase in SI - ~ 3.8-fold was found in day 45 animals. Surprisingly, a decrease was observed in day 90 mice exposed to both – low and high doses. Significant differences between both compounds were found for days 30 and 45.

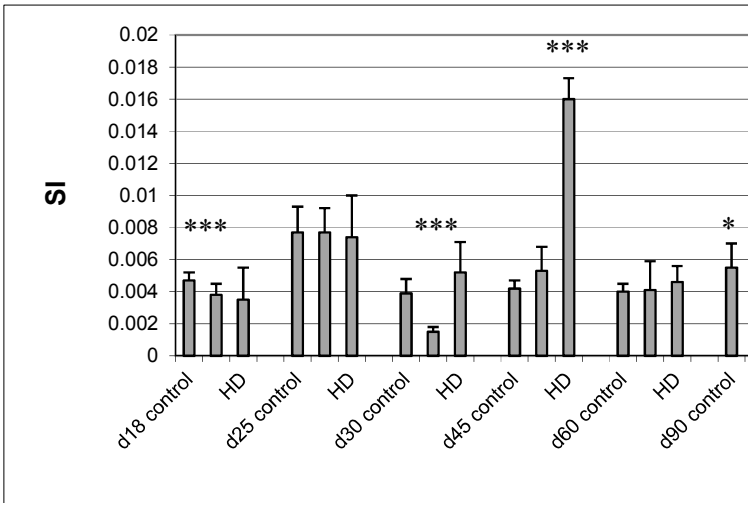

Fig. 2. Spleen index (SI) of mice treated with high dose (125 mg/kg b.w.) $CoCl_2$ and Co-EDTA. "HD" represents high dose Co-EDTA. Each column represents mean±SD, *n* = 5. Asterisk (*) represents statistical difference ($p<0.01$) and triple asterisk (***) represents statistical difference ($p<0.001$).

These changes could be explained by the alterations in extramedullary hematopoiesis as well as the significant accumulation of Co ions as previously shown by us [6].

Our results are in agreement with Simonyte et al. [10] and Dkhil [4] demonstrating increased SI in mice after long-term exposure to heavy metals and infections. Increased SI is associated with changes both in the white and red pulp [4].

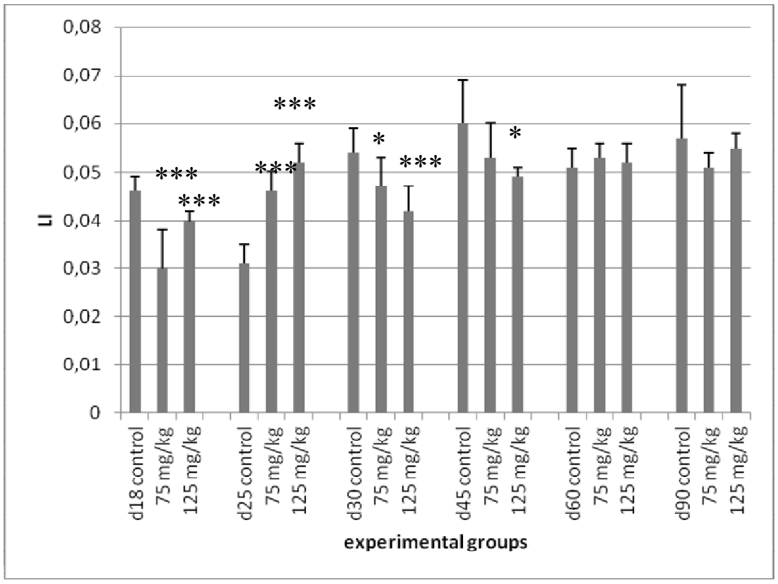

Fig. 3. Liver index (LI) of mice treated with $CoCl_2$. Each column represents mean±SD, n = 5. Asterisk (*) represents statistical difference ($p<0.01$) and triple asterisk (***) represents statistical difference ($p<0.001$).

$CoCl_2$ decreased LI in all experimental groups except in day 25 mice (Fig. 3). In day 25 animals LI significantly increased compared to untreated controls. The index of mice treated with the high dose was significantly higher ($p<0.01$) compared to that of mice exposed to the lower dose.

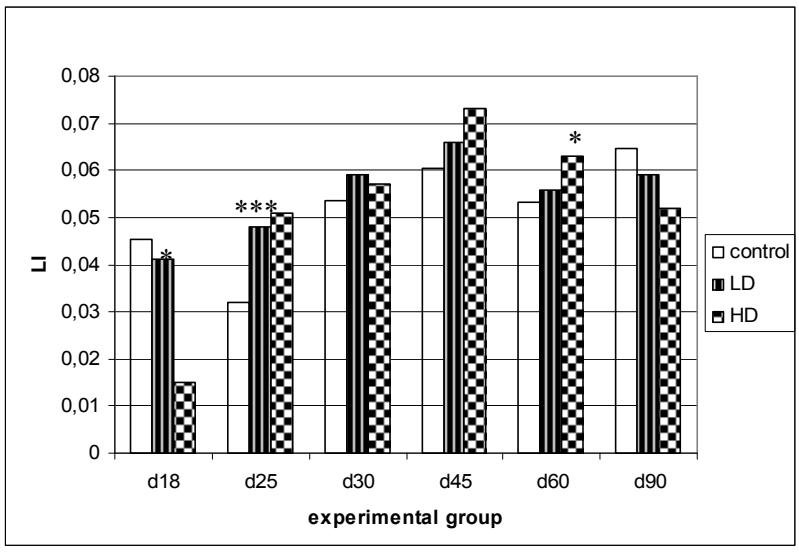

Fig. 4. Liver index (LI) of mice treated with Co-EDTA. Each column represents mean±SD, n = 3. Asterisk (*) represents statistical difference ($p<0.01$) and triple asterisk (***) represents statistical difference ($p<0.001$).

Exposure to Co-EDTA led to a 1.2 to 1.6-fold increase in LI (Fig. 4). The tendency was observed in all experimental groups except day 18 and day 90 mice. The reduced LI in day 18 experimental animals could be explained with the fact that they are more sensitive to treatment. The results suggest possible hepatotoxicity of $CoCl_2$ as demonstrated by Liu et al. [8] in mice intraperitoneally injected with $CoCl_2$. Garoui et al. [5] show decreased liver weight in day 14 rats. Our results also showed reduced weight for day 18 mice but increased index. We suggest that changes in liver index should be considered as additional marker for accurate evaluation of the biological effects of different compounds on the liver instead of only organ weight.

Treatment with $CoCl_2$ increased KI in immature animals as well as in day 60 mice (Fig. 5).

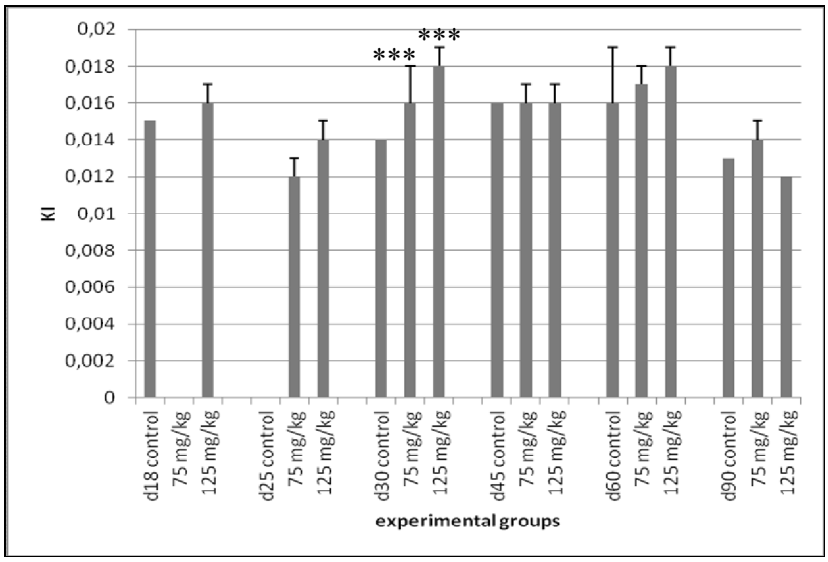

Fig. 5. Kidney index (KI) of mice treated with $CoCl_2$. Each column represents mean±SD, $n = 5$. Triple asterisk (***) represents statistical difference ($p<0.001$).

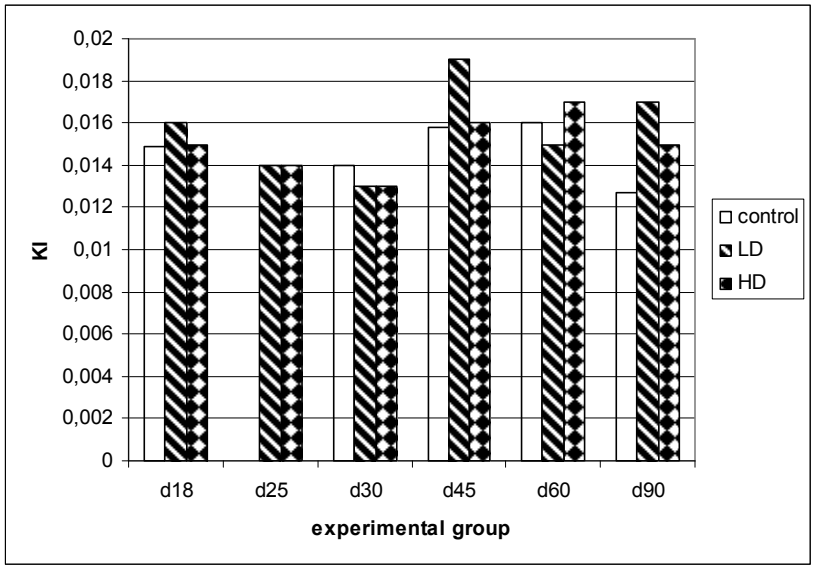

Fig. 6. Kidney index (KI) of mice treated with Co-EDTA.

Kidney index (KI) was increased (~1.1 to 1.3-fold) in mature mice after exposure to Co-EDTA compared to untreated controls (Fig.6).

CONCLUSIONS

The results indicate that the biological effects of Co(II) depend on the type of compound, the duration of exposure and as well as on the age of the experimental animals. Co-EDTA applied in low or high dose increased SI of mature mice. Both compounds exhibited diverse effects on liver - $CoCl_2$ decreased LI in almost all experimental groups while Co-EDTA increased the index except for d18 and d90 animals. $CoCl_2$ increased KI in immature and d60 mice but no clear tendency was observed for Co-EDTA. Alterations in organ weight indices could be an additional marker for diagnosing metal intoxications.

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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, Vol. 56, 1999, 571-591.

2. Barceloux D.G., D. Barceloux, Cobalt, Clin. Toxicol., Vol. 37, 1999, 201-216.

3. Cesta M., Normal structure, function, and histology of the spleen, Toxicol. Pathol., Vol. 34, 2006, 455-465.

4. Dkhil M., Apoptotic changes induced in mice splenic tissue due to malaria infection, J. Microbiol. Immunol. Infect., Vol. 42, 2009, 13-18.

5. 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., Vol. 63, 2009, 9-15.

6. Gluhcheva Y., V. Atanasov, Ju. Ivanova, M. Mitewa, Cobalt-induced changes in the spleen of mice from different stages of development, J. Toxicol. Environ. Health A, Vol.75, 2012, 1418–1422.

7. Jelkmann W., The disparate roles of cobalt in erythropoiesis, and doping relevance, Open J Hematol., Vol. 3, 2012, 1-9

8. 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, Vol. 107, 2006, 698-707.

9. Nangaku M., K.-U. Eckardt, Hypoxia and the HIF system in kidney disease, J. Mol. Med., Vol. 85, 2007, 1325–1330.

10. Simonyte S., R. Planciuniene, G. Cherkashin, G. Zekonis, Influence of long-term cadmium and selenite exposure on resistance to Lysteria monocytogenes during acute and chronic infection in mice, Biologija, Vol. 3, 2006, 92-95.

ВЛИЯНИЕ НА ХРОНИЧНОТО ТРЕТИРАНЕ С КОБАЛТ (II) СЪЕДИНЕНИЯ ВЪРХУ ТЕГЛОВНИТЕ ИНДЕКСИ НА ОРГАНИТЕ

Й. Глухчева, М. Маджарова, Ю. Иванова

Резюме. Широкото приложение на кобалта (Co) в индустрията, медицинските прибори, като консервант, в козметиката налага детайно изследване на неговите биологични ефекти. Целта на изследването е да се проучи влиянието на хроничното третиране с кобалт(II) съединения – кобалтов хлорид ($CoCl_2$) и кобалт-ЕДТА (Co-EDTA) върху тегловните индекси на органите на полово зрели и полово незрели мишки. Бременни мишки – линия ICR са третирани всеки ден с 75 mg/kg телесна маса или 125 mg/kg телесна маса $CoCl_2$ или Co-EDTA до 90-дневна възраст на новородените мишлета. Съединенията на кобалта са разтворени в чешмяна вода. Контролните животни приемат чиста чешмяна вода, а всички мишки имат достъп до храна *ad libitum*. След навършване на 25-дневна възраст новородените мишки са отделени в самостоятелни клетки и третирането продължава до 90-ти ден от раждането на мишлето. Всяка седмица е проследявана промяната в теглото на мишките, за коригиране на дозите. През различни периоди – 18-, 25-, 30-, 45-, 60- и 90-ти ден експерименталните животни са изследвани. Слезката, черният дроб и бъбреците на мишките са отпрепарирани, премерени и са изчислени тегловните индекси на органите – на слезката (SI), на черния дроб (LI) и на бъбреците (KI). Хроничният прием на Co(II) съединения предизвиква промени и в тегловните индекси изследваните органи - SI, LI, KI. $CoCl_2$ повишава SI при полово незрелите мишки, докато Co-EDTA повлиява слезката при полово зрелите. Третирането с Co повишава LI на мишките, приемали Co-EDTA. Този индекс е

понижен в случаите на приемане на $CoCl_2$. Тегловният индекс на бъбреците (KI) е значително повишен при 30-дневните мишки след третиране с $CoCl_2$. Същото съединение повлиява слабо KI при полово зрелите животни. Влиянието на Co-EDTA върху KI е разнопосочно без ясно изразена тенденция. Резултатите показват, че влиянието на Co(II) зависи от вида на съединението, продължителността на третиране и от възрастта на експерименталните животни.

Ключови думи: мишки, ин виво модел, кобалтов хлорид, кобалт-ЕДТА, тегловни индекси на органите

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