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COBALT(II)-INDUCED CHANGES IN HEMOGLOBIN CONTENT AND IRON CONCENTRATION IN MICE FROM DIFFERENT AGE GROUPS

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ABSTRACT

Cobalt (Co) and its compounds are shown to improve hematological parameters. Long-term treatment with Co(II) increased hemoglobin content in a dose- and time-dependent manner in mature mice (day 45 to day 90) while it was reduced in immature mice (day 18 to day 30). Higher Hb was measured in samples treated with $CoCl_2$ compared to those treated with Co-EDTA. Plasma Fe concentration was significantly higher in samples treated with Co-EDTA compared to those exposed to $CoCl_2$. Lower concentrations were measured only in mature animals. Co(II) concentration increased but not in a dose-dependent manner. In general more Co(II) was measured in samples treated with $CoCl_2$ possibly due to the stability of the complex Co-EDTA. Surprisingly, mature mice had less Co(II) in their plasma compared to day 18 mice. Strong correlation between plasma Co(II) and iron concentration was found in samples of mice treated with Co-EDTA. Co(II) concentration showed inverse correlation with hemoglobin in mice treated with low dose Co-EDTA. Such relationship was found for day 45 and day 60 mice exposed to high dose $CoCl_2$. Immature mice are more sensitive to Co(II) treatment and show signs of anemia. It has a significant impact on hemoglobin biosynthesis possibly due to its effect on iron metabolism.

Keywords: cobalt(II) compounds, haemoglobin, plasma iron

Introduction

Cobalt (Co) is a trace element present almost in all living organisms. Its compounds are shown to improve hematological parameters by increasing bone marrow erythropoietic activity, hematocrit and mean cell volume (2). Topashka-Ancheva et al. determined that consuming food containing industrial dust with cobalt induces changes in hemoglobin, hematocrit, in red and white blood cell counts (6, 7). Data suggest a relationship between plasma cobalt and iron concentrations (1, 8). Cobalt also binds to plasma transferrin and forms a stable complex. Thus, cobalt competes with iron for incorporation in the heme moiety (5). There are lack of data for the effect of long-term treatment with Co(II) compounds on mice from different stages of development. The aim of the present work is to study the effect of water-soluble cobalt(II) compounds ($CoCl_2$ and Co-EDTA) on hemoglobin content, iron concentration and Co(II) accumulation in plasma of mice from different age groups.

Materials and Methods

Animal model

Pregnant balb/c mice in late gestation were subjected to cobalt chloride ($CoCl_2.6H2O$) or Co-EDTA treatment at daily doses of 75 mg/kg or 125 mg/kg until day 90 of the newborn pups. Cobalt(II) compounds were obtained from drinking tap water. 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 to ensure that all experimental animals obtained the required dose $CoCl_2$. The experimental animals were weighed weekly and the experimental cobalt concentration was adjusted accordingly. The newborn pups were sacrificed on days 18, 25, 30, 45, 60 and 90 which correspond to different stages of development.

Biochemical analysis

Whole blood samples were obtained, centrifuged and plasma was stored at -20°C until further analysis. Plasma samples were used for measuring the amount of cobalt(II), hemoglobin (Hb) content and iron (Fe) concentration. The amount of cobalt in plasma was measured using electro-thermal atomic absorption spectrometry (ET-AAS) using Zeeman Perkin Elmer 3030 instrument, HGA 600, pyrolitic coated graphite tube as atomizer. Hb content was determined by hemiglobincianide method (HiCN) (4). Plasma iron concentration (transferrin-bound iron) was measured using "Iron Liquid" analytical kit based on Ferene-S as a chromogen (Sentinel Diagnostics, Italy).

Statistical analysis

The obtained results are presented as mean value ± SD. Statistical significance between the experimental groups was determined using Student's *t*-test. Difference was considered

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significant at $p<0.05$. Correlation between parameters was analyzed using simple correlation coefficient **r**.

Results and Discussion

Long-term treatment with Co(II) increased hemoglobin content (**Fig.1**, **Fig 2**) in a dose- and time-dependent manner in mature mice (day 45 to day 90) while it was reduced in immature mice (day 18 to day 30) compared to control animals. Higher Hb was measured in samples treated with $CoCl_2$ compared to those treated with Co-EDTA.

Results show that high dose $CoCl_2$ has a better effect on haemoglobin content compared to the low dose.

The results indicate that when Co-EDTA is used longer treatment is necessary to achieve the same effect as that of $CoCl_2$. Immature animals are more sensitive to Co(II) exposure compared to adults showing signs of anemia compared to age-matched controls. Red blood cell count is also necessary to evaluate the effect of long-term treatment with cobalt compounds on erythropoiesis.

Plasma Fe concentration was significantly higher in samples treated with Co-EDTA compared to those exposed to $CoCl_2$. Lower concentrations were measured only in mature animals (**Fig. 3**, **Fig. 4**). The elevated plasma iron level corresponded to the lower haemoglobin content measured in the same samples.

Plasma Co(II) concentration increased but not in a dose-dependent manner. In general more Co(II) was measured in samples treated with $CoCl_2$ possibly due to the stability of the complex Co-EDTA (**Fig. 5**, **Fig. 6**). Results indicate that the solubility of the compounds is an important factor for bioaccumulation in plasma and in the organs respectively. Surprisingly, mature mice had less Co(II) in their plasma compared to day 18 mice. The result is in agreement with WHO report according to which young animals accumulate more cobalt(II) than adults (8). Comparison between the high dose $CoCl_2$ and Co-EDTA showed statistically significant differences in plasma cobalt concentration for day 25 ($p<0.001$), 30 ($p<0.001$) and 60 mice ($p<0.01$).

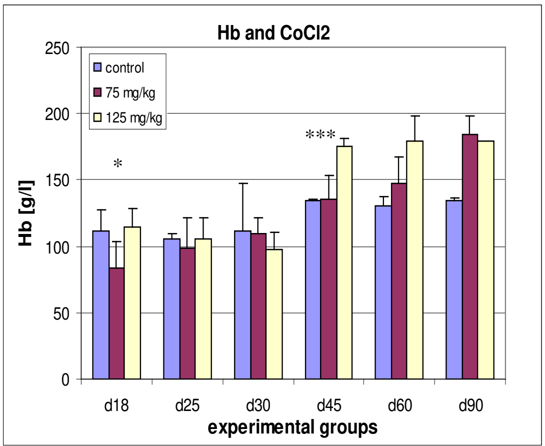

Fig. 1. Hemoglobin content in mice treated with $CoCl_2$
*$p<0.05$, ***$p<0.001$

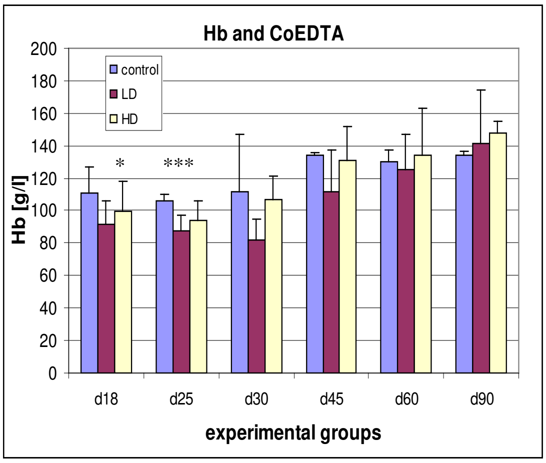

Fig. 2. Hemoglobin content in mice treated with Co-EDTA
*$p<0.05$, ***$p<0.001$

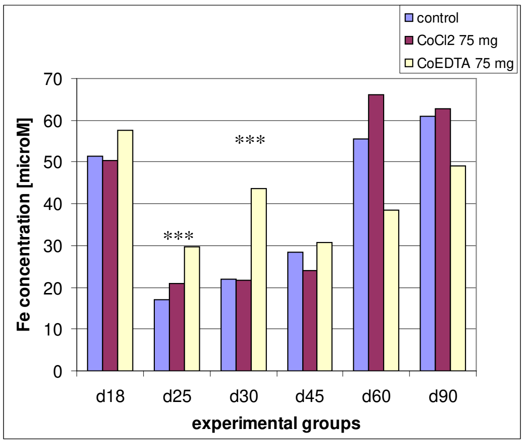

Fig. 3. Plasma iron concentration in mice treated with low dose $CoCl_2$ or Co-EDTA
*$p<0.05$, ***$p<0.001$

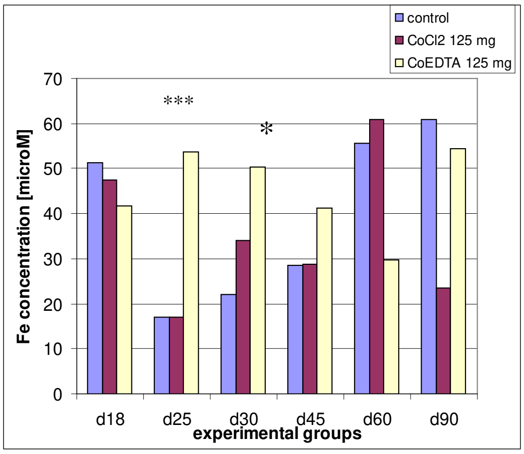

Fig. 4. Plasma iron concentration in mice treated with high dose $CoCl_2$ or Co-EDTA
*$p<0.05$, ***$p<0.001$

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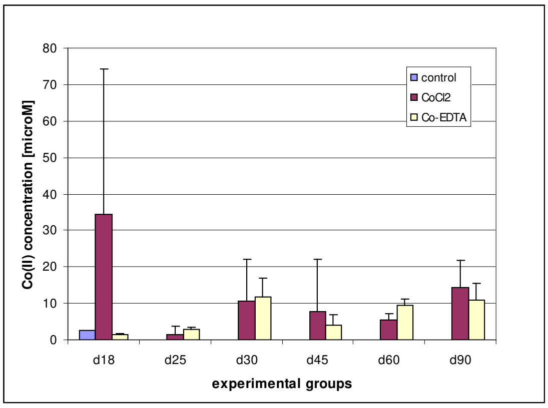

Fig. 5. Plasma Co(II) concentration in mice treated with low dose $CoCl_2$ and Co-EDTA

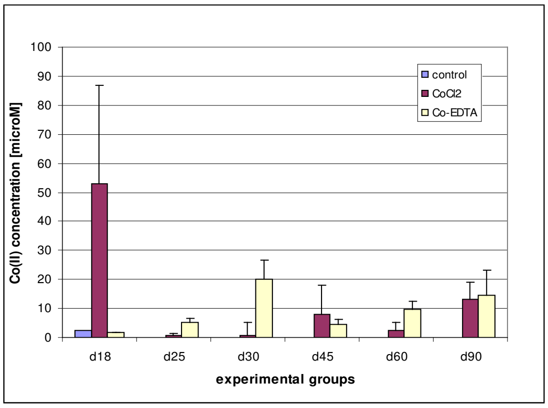

Fig. 6. Plasma Co(II) concentration in mice treated with low dose $CoCl_2$ and Co-EDTA

Strong correlation between plasma Co(II) and iron concentration was found in samples of mice treated with Co-EDTA. Results are in agreement with those of Barany et al. (1) showing that iron status affects cobalt concentration in blood plasma. The ions of both metals are shown to compete with close affinity constant for transferrin (3). It is further necessary to analyze Co(II) concentration bound to transferrin as well as the amount of the element within the erythrocytes in order to understand how much cobalt(II) was incorporated in the cells. This could help elucidate the increased plasma iron concentration. On the other hand, Co(II) concentration showed inverse correlation with hemoglobin in mice treated with low dose Co-EDTA. Such relationship was found for day 45 (r=-0.7) and day 60 (r=-0.6) mice exposed to high dose $CoCl_2$.

The results indicate that among immature mice day 18 mice are more sensitive to treatment with Co(II) compounds. They showed the lowest haemoglobin content and the highest plasma Co(II) concentration.

Conclusions

The effect of chronic exposure to cobalt(II) depends on the type of compound used, dose, time duration as well as on the age of the experimental animals. Immature mice are more sensitive to Co(II) treatment and show signs of anemia. Cobalt(II) has a significant impact on hemoglobin biosynthesis possibly due to its effect on iron metabolism.

Acknowledgements

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