EFFECT OF COBALT-EDTA ON IRON CONTENT IN SPLEEN AND LIVER OF IMMATURE MICE

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Cobalt (Co) is an essential trace element and its accumulation affects the concentrations of other elements also. Co(II) is shown to compete with iron (Fe) for the transferrin receptor and to form a stable complex with haemoglobin thus affecting haematopoiesis. There are lack of data regarding the effect of chronic exposure to Co compounds on Fe content in spleen and liver of mice. The study deals with the effect of long-term treatment with cobalt-EDTA (Co-EDTA) on iron content in the spleen and liver of immature mice. Pregnant ICR mice were subjected to chronic treatment with daily dose of 75 mg/kg Co-EDTA which continued until day 25pnd of the newborn pups. Results show accumulation of Co(II) and altered Fe content in the spleen and liver of treated mice compared to age-matched controls with significantly increased Fe concentration in the livers of treated mice. The changes could explain impaired haematopoiesis and immune responses of exposed to Co(II) immature mice.

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Introduction

Cobalt (Co) is an essential trace element and its accumulation in blood plasma and other organs affects the concentrations of other elements[1]. The wide use of cobalt alloys in medical devices requires full elucidation of its biological role in cells, tissues and organs after long-term exposure[2,3]. Co(II) is shown to compete with iron (Fe) for the transferrin receptor and to form a stable complex with haemoglobin[4] thus affecting haematopoiesis. Barany et al. found higher concentrations of Co in blood at low body iron storrages[5].

Iron is another essential micronutrient required for erythropoietic function, DNA synthesis, oxidative metabolism and cellular immune response[6,7]. Spleen and liver are the main sites of iron storage in the body.

There are lack of data regarding the effect of chronic exposure to Co compounds on Fe content in spleen and liver of mice. Data show that cobalt is transferred from food into human milk[8]. Added cobalt caused an increase in cobalt concentration in the milk during late gestation and early lactation in cows[9]. According to Shingfield et al. ruminal infusion of Co-EDTA alters milk fatty acid composition in lactating cows[10].

The aim of the present study is to investigate the effect of long-term treatment with cobalt-EDTA (Co-EDTA) on iron content in the spleen and liver of immature mice.

Methods

Synthesis of Co-EDTA complex

All chemicals and solvents used were of AR grade. Co-EDTA was synthesized according to modified literature procedures[11,12] as already described[13].

In vivo animal model

Pregnant ICR mice in late gestation were subjected to cobalt EDTA (Co-EDTA) treatment at daily doses of 75 mg/kg that continued until day 25 of the newborn mice. Cobalt compound was dissolved and obtained from drinking tap water. Each pregnant mouse received 1.7 mg Co-EDTA dissolved in 8 ml tap water. Our studies have shown that this is the amount of water that an adult mouse drinks per day. Pure tap water was used as control. 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.

The newborn pups (n=3 per group) were sacrificed on days 18 and 25. Mice were weighed weekly and the experimental cobalt concentration was adjusted accordingly. Spleen and liver were excised and used for measuring cobalt bioaccumulation and Fe content.

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

Analysis of cobalt content in spleen and liver

Cobalt and iron contents in the spleen and liver were determined after nitric acid wet digestion by flame atomic absorption spectrometry (FAAS) using Perkin Elmer AAnalyst 400, flame: air – acetylene.

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 significant at $p<0.05$.

Results

Since cobalt is transferred from food into the mother's milk the newborn pups were exposed to it as well. Co concentration in the milk was not measured in this study. The pups showed no differences regarding number and viability when compared to control ones. Reduction in food or water consumption was not observed and all animals survived the experiment.

Preliminary results indicate that chronic exposure of immature mice to Co-EDTA increased Co(II) content in the spleen and liver of treated animals (Figs. 1 and 2).

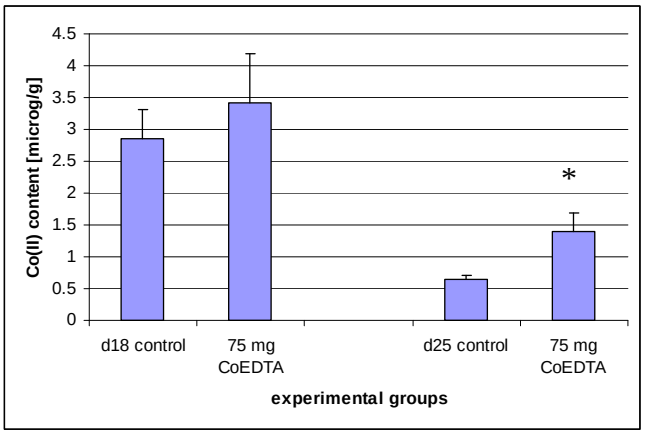

Figure 1. Cobalt(II) content in the spleen of experimental animals. Each column represents mean±SD, n = 3. Asterisk (*) represents statistical differences ($p<0.05$).

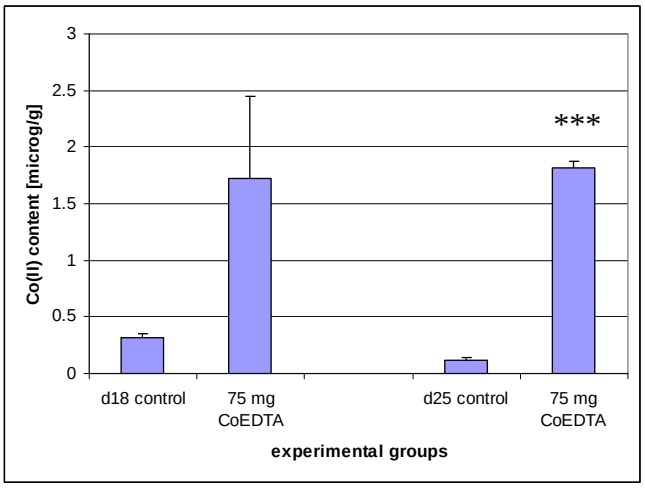

Figure 2. Cobalt(II) content in the liver of experimental animals. Each column represents mean±SD, n = 3. Triple asterisk (***) represents statistical differences ($p<0.001$).

Livers of treated mice accumulated up to ~ 15-fold Co(II) compared to untreated controls. Spleens accumulated less which indicates that liver is more sensitive to Co(II) exposure than spleen.

The Co(II) measured in the organs of the control mice could be due to cobalt containing supplements in the food they obtained.

Exposure to Co-EDTA induced changes in the iron content as well. Surprisingly, spleen of day18 Co-EDTA-treated mice had less Fe compared to the controls (Fig.3).

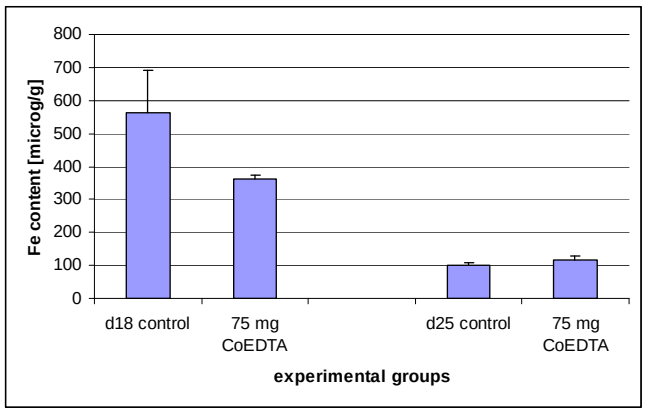

Figure 3. Iron content in the spleen of experimental animals. Each column represents mean±SD, n = 3.

Iron was significantly increased in the liver of treated mice compared to age-matched controls (Fig.4).

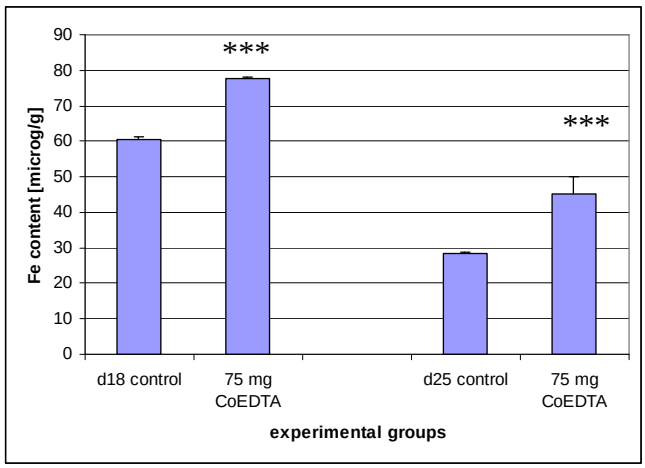

Figure 4. Iron content in the liver of experimental animals. Each column represents mean±SD, n = 3. Triple asterisk (***) represents statistical differences ($p<0.001$).

Discussion

As inorganic and complex compounds (with organic ligand) cobalt(II) is used as nutritional supplement, preservative, in drinks, as therapeutic agent for treating different diseases, etc. Co(II) compounds are shown to induce DNA damage, gene mutations, sister chromatid exchanges and aneuploidy in *in vitro* studies on animal and human cells[14]. On the other hand, treatment with EDTA

alone has shown to induce severe biochemical and histopathological changes in bone marrow, liver, kidneys and testes of treated rats[15]. The compound led to a higher incidence of micronucleated polychromatic erythrocytes and chromosome aberrations in bone marrow cells in mice and Syruan hamster embryo cells[15,16]. The lowest EDTA dose reported to cause a toxic effect in animals was 750 mg/kg/day. The dose of Co-EDTA used in the present study is 75 mg/kg/day or 10-fold less and toxic effects on the experimental animals should not be expected.

In our studies chronic exposure to Co-EDTA resulted in accumulation of Co(II) ions in the spleen and liver of treated mice which affected iron content as well. The results are in good agreement with WHO report that spleen and liver are sensitive to cobalt treatment and accumulate the metal ions[17]. Korf et al. also show significant uptake of radioactive cobalt in rat spleen 24h after injection[18].

Under physiological conditions there is a balance between iron absorption, transport and storage in the human body[7]. TfR1 and TfR27 mediate the uptake of transferrin-bound Fe by the liver from blood plasma.

Surprisingly, day18 mice had less Fe in their spleen compared to the controls. This is possibly the reason for the decreased haemoglobin content and other erythrocytic indices of the treated mice (data not shown). Hypoxia increases iron storage in the spleen[19]. Day 25 mice show increased Fe content in their spleen that may be due the prolonged treatment and the effect of hypoxia induced by Co-EDTA. The results could explain our previous observation about disturbed extramedullar haematopoiesis in the spleen (reduced number of megakaryocytes), after long-term treatment of mice with low (75 mg/kg) or high (125 mg/kg) dose Co-EDTA[20].

Liver plays a key role in body's detoxification which explains the high amount of Co(II) accumulated. Hepcidin, produced mainly by the haepatocytes is responsible to liver iron levels, inflammation, hypoxia and anemia and is the key Fe regulatory hormone for iron absorption and recirculation[7]. Anemia and hypoxia trigger a decrease in hepcidin levels[21]. Cobalt is a hypoxia inducing agent and this could explain the increased Fe content in the liver due to suppressed hepcidin levels. Garoui et al. find that exposure of rats to cobalt chloride during pregnancy and early postnatal periods affects antioxidant enzyme activities and lipid peroxidation in the liver of treated mothers and their offspring[22]. Possible effects may be suggested for Co-EDTA as well.

Co-EDTA treatment affects early postnatal mouse development altering Fe homeostasis. Administration of the complex in the animal feed requires full elucidation of its effects on key physiological processes.

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